TECHNICAL SUPPORT DOCUMENT FOR CANCER POTENCY FACTORS

APPENDIX J

In Utero and Early Life Susceptibility to Carcinogens: The Derivation of Age-at-Exposure Sensitivity Measures

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In Utero and Early Life Susceptibility to Carcinogens:

The Derivation of Age-at-Exposure Sensitivity Measures

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California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Branch

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Executive Summary

Early-in-life susceptibility to carcinogens has long been recognized by the scientific community and clinicians as a public health concern. Numerous scientific publications and symposia have addressed this issue over the years and the scientific literature contains a number of human clinical findings and epidemiological studies of early life cancer susceptibility. While there are many indications of increased human cancer susceptibility in early life, the magnitude of the impact has been difficult to gauge. Until recently risk assessment procedures have not in general addressed the issue. The California legislature in 2000 recognized the need for a systematic approach to develop scientifically based methods to address this concern so that in environmental decision making special sensitivities of the developing fetus, and the young were taken into account. The legislature directed the Office of Environmental Health Hazard Assessment (OEHHA) to assess methodologies used in addressing early-in-life risk, compile animal data to evaluate those methods, and develop methods to adequately address carcinogenic exposures to the fetus, infants, and children (Children's Environmental Health Initiative [AB 2872, Shelly]; California Health and Safety Code [HSC] section 901 [a] through [e]).

In 2001, OEHHA assessed cancer risk assessment methodologies, and concluded that the existing risk assessment approaches did not adequately address the possibility that risk from early-in-life exposures may differ from that associated with exposures occurring in adulthood. OEHHA further concluded that there was a need for methodologies addressing early-in-life cancer risk to be developed, tested, and validated.

Also in 2001, OEHHA began compiling animal cancer studies with early life exposure to carcinogens, as a first step toward developing methods to address early-in-life cancer risk. Two types of studies with early-in-life carcinogen exposures were compiled. The first type are "multi-lifestage exposure studies." These studies have at least two groups exposed during different lifestages. The prenatal lifestage is defined as the period from conception to birth, the postnatal lifestage is defined as the period from birth to weaning, the juvenile lifestage is defined as the period from weaning to sexual maturity, and the adult lifestage is defined as beginning at the time of sexual maturity. One dose group is exposed to a chemical only during one early lifestage (prenatal, postnatal, or juvenile). The second dose group is exposed for some period of

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time at an older age, preferably during the adult lifestage. This group serves as the referent group. In some cases where there was no adult exposure group, animals exposed as juveniles served as the referent group. Multi-lifestage exposure studies are available for many carcinogens, enabling the exploration of patterns in early-life susceptibility across chemicals.

The second type are "single-lifestage exposure experiments." In these "single-lifestage exposure experiments" dose groups were exposed to a carcinogen only during a particular lifestage and, unlike the "multi-lifestage exposure studies," there was no requirement that the same study also include groups exposed during a different lifestage. Thus, single-lifestage exposure experiments were identified as being either prenatal, postnatal, juvenile, or adult exposure studies. OEHHA conducted "chemical-specific case studies" of early-life sensitivity for two specific carcinogens, ethyl-N-nitrosoamine (DEN) and N-ethyl-N-nitrosourea (ENU). For each of the two chemicals, there were many prenatal studies conducted that were compiled, analyzed, and grouped together. Postnatal studies from different publications were similarly compiled, analyzed and grouped together, as were juvenile studies. Adult studies were not available for either DEN or ENU, thus for both chemicals juvenile exposure studies served as the referent for prenatal studies, and for postnatal studies. These "chemical-specific case studies" were conducted to explore the feasibility of analyzing chemical-specific data on age susceptibility from single-lifestage exposure experiments.

This document presents 1) the statistical methods developed and used to systematically analyze the data from multi-lifestage exposure studies and single-lifestage exposure experiments to derive measures of early-life susceptibility; 2) the results of applying these analyses to multi-lifestage exposure studies on 23 unique carcinogens and two chemical-specific case studies of single-lifestage exposure experiments on diethylnitrosamine (DEN) and ethylnitrosourea (ENU); and 3) conclusions regarding the sensitivity of the fetus, infants, and children to carcinogen exposures.

Analytical Approach

Analysis of the data involved the derivation of a cancer potency, that is, the slope of the dose response curve, for each of the experiments selected, using the linearized multistage model. This model was chosen because of widespread use in risk assessment, and its flexibility in being able

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to fit many different data sets needed to evaluate the effect of lifestage-at-exposure on cancer potency. To take into account uncertainty in potency estimation, cancer potencies are depicted by a statistical distribution, generated using profile-likelihood methods, rather than by a single, fixed value.

An "experiment" was defined as a study component consisting of a control group as well as a treated group(s) exposed during the same lifestage and using the same experimental protocol (e.g., route of exposure, strain, species, laboratory). When treatment-related tumors were observed at multiple sites in an experiment, or at the same site, but arising from different cell types, slopes from these different sites or types were statistically combined to create an overall multisite cancer potency for that experiment. It is not uncommon that a carcinogen causes more than one type of cancer or causes tumors at different sites depending on lifestage at exposure. In order to account for this, all treatment-related tumors that were observed in a given lifestage were taken into account in estimating cancer potency from that particular experiment.

OEHHA calculated the ratio of cancer potency derived from an early lifestage exposure experiment to that derived from an experiment conducted in adult animals, referred to here as a lifestage potency (LP) ratio. OEHHA used the potency distributions for the individual lifestage exposures, rather than a point estimate, to derive the ratios. The lifestage cancer potency ratio is then described as a distribution and one can select specific percentiles from the distribution to better understand and bound the uncertainty.

A lifestage potency (LP) ratio distribution was derived for each multi-lifestage exposure study, resulting in 22 prenatal ratio distributions representing 14 unique carcinogens, 55 postnatal LP ratio distributions representing 18 unique carcinogens, and seven juvenile LP ratio distributions representing five unique carcinogens. The LP ratio distributions for a given early lifestage were combined into a single "LP ratio mixture distribution," in order to show the range of susceptibilities of that lifestage to the carcinogens studied.

LP ratio mixture distributions for a given early lifestage were developed by (1) obtaining a single LP ratio distribution for each chemical (when a chemical is represented by more than one study) and then (2) equally sampling across all chemicals. When a chemical is represented by more

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than one study, then the LP ratio distributions from all studies of that chemical were combined by equally sampling from each LP ratio distribution via Monte Carlo methods to obtain a single LP ratio distribution for that chemical. Sensitivity analyses were also conducted, employing alternative sampling methods to obtain a single LP ratio distribution to represent each chemical for which there were multiple studies. Once each chemical is represented by a single LP ratio distribution, then the LP ratio mixture distribution for each early lifestage (prenatal, postnatal, and juvenile) is obtained by equally sampling across all of the chemicals via Monte Carlo methods.

The LP ratios characterize the inherent susceptibility of early lifestages to carcinogen exposure, by comparing potencies for individuals followed for similar periods of time and similarly exposed, but exposed during different lifestages. Cancer risk increases with age, or time since first exposure, and age-specific adjustments to the cancer potency must also take this into account. Thus, consistent with the approach used by the National Toxicology Program (NTP) in analyzing rodent cancer bioassay data, the longer period of time that exposed young have to develop tumors is addressed by taking into account time-of-dosing, and assuming that cancer risk increases by the third power of age. This was done by multiplying the LP ratio by a time-of-dosing factor, to yield an age sensitivity factor (ASF). Specifically, the prenatal LP ratio is multiplied by a factor of 3.0, the postnatal LP ratio is multiplied by a factor of 2.9, and the juvenile LP ratio is multiplied by 2.7. Thus, the ASF calculated for carcinogens includes both inherent sensitivity of developing animals and the available time since exposure to develop cancer.

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Characteristics of the Chemicals Studied

Twenty of the 23 carcinogens included in the multi-lifestage exposure studies analyses are considered to act via primarily genotoxic modes of action, with 15 thought to require metabolic activation to the ultimate carcinogenic species. Fourteen carcinogens, including one thought to act via primarily nongenotoxic modes of action, were included in the prenatal multi-lifestage exposure studies. Eighteen carcinogens, including two thought to act via primarily nongenotoxic modes of action, were included in the postnatal multi-lifestage exposure studies. Five carcinogens were included in the juvenile multi-lifestage exposure studies. The chemical-

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specific case study chemicals, DEN and ENU, are both genotoxic. ENU is a direct acting alkylating agent, while DEN requires metabolic activation.

Results

The results of the multi-lifestage exposure studies and chemical-specific case study analyses indicate that the prenatal, postnatal, and juvenile lifestages can be, but are not always, much more susceptible to developing cancer than the adult lifestage. While there is a great deal of variability across the chemicals studied in the prenatal ASFs, the potency associated with prenatal carcinogen exposure is not zero. The median estimate of the prenatal ASF mixture distribution from the multi-lifestage exposure studies analysis was 2.9, and the mean estimate was 21.0. The DEN and ENU case studies illustrate the variability across chemicals in the sensitivity of the prenatal period, with results for DEN suggesting inherently less sensitivity than older animals from *in utero* exposure, and for ENU just the opposite. ENU does not require metabolic activation, whereas DEN does and cannot be metabolized to any significant extent by fetal tissues until relatively late in gestation. This may explain the lower fetal susceptibility of DEN. However, the multi-lifestage exposure studies illustrate that *in utero* metabolic status is not the sole determinant of *in utero* susceptibility: benzidine and safrole require metabolic activation and exhibit greater susceptibility from *in utero* exposure.

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In the case of exposures occurring during the postnatal lifestage, the data indicate an inherently greater susceptibility compared to the adult lifestage. The median estimate of the postnatal LP ratio mixture distribution was 4.6 and the mean estimate was 27.1. The median estimate of the postnatal ASF mixture distribution from the multi-lifestage exposure studies analysis was 13.4 and the mean estimate was 78.5. The DEN and ENU case studies also exhibit substantial sensitivity during the postnatal lifestage.

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While there were just five chemicals and seven studies, two of which were not independent, available to examine susceptibility in the juvenile lifestage, significantly greater susceptibility compared to the adult lifestage was observed in three of the six independent studies. The median estimate of the juvenile ASF mixture distribution from the multi-lifestage exposure studies analysis was 4.5 and the mean estimate was from 7.1.

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The multi-lifestage exposure studies and chemical-specific case studies exhibited considerable variability across carcinogens in age-at-exposure related susceptibility. There is also variability in age-at-exposure related susceptibility among studies of the same carcinogen. The sources of variability evident in the analyzed studies include timing of exposure within a given lifestage, and gender, strain, and species differences in tumor response. The set of studies identified and analyzed in this document was not sufficiently robust to fully describe quantitatively the variability. This variability raises concerns that selection of the median, that is the 50th percentile, estimates for lifestage-specific ASFs may considerably underestimate effects for certain carcinogens or population groups. Relatively large variability in humans in response to carcinogens is expected to be common (Finkel, 1995; 2002).

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Discussion

Taken together, these results indicate that early lifestages are generally more sensitive to carcinogen exposure than adults, and that cancer risk assessment practices should take increased sensitivity of the young into account. When data on age-at-exposure related susceptibility are lacking for a specific carcinogen, these analyses indicate that increased susceptibility of the young is a scientifically justifiable assumption. This early-life susceptibility can be addressed by applying adjustments such as ASFs to the adult cancer potency slope factor when estimating cancer risk associated with early life exposures.

Table 1 illustrates the effect of lifestage-specific ASFs on lifetime cancer risk. In this example, exposure to the carcinogen is assumed to occur at a constant exposure rate over the entire lifetime. Risk calculations were performed using the mean, 50th, 70th, and 95th percentile ASF values. As shown in Table 1, when increased susceptibility of the fetus, infants, and children is taken into account by applying 50th percentile ASF values, the total lifetime cancer risk is increased two-fold; applying 70th percentile ASF values increases the estimate three-fold, applying mean ASF values increases the estimate nearly five-fold, and applying 95th percentile ASF values increases the estimate 16-fold above the risk estimated in the absence of age-specific adjustments to the potency.

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Table 1. Comparison of cancer risk estimates[1] for lifetime exposure to 0.0001 mg/kg-d of a carcinogen with potency 1 (mg/kg-d)$^{-1}$ based on different parameters of ASF distributions, or U.S. EPA values.

Lifestage	Years of life exposed	No adjustment		50th percentile		70th percentile		Mean		95th percentile		U.S. EPA (2005)	
		ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	Factor	Risk
In utero	0.75	0	0.0	3	3.2×10^{-6}	10	1.1×10^{-5}	21	2.2×10^{-5}	115	1.2×10^{-4}	0	0.0
Birth to <2 yr	2	1	2.9×10^{-6}	13	3.7×10^{-5}	28	7.9×10^{-5}	79	2.3×10^{-4}	350	1.0×10^{-3}	10	2.9×10^{-5}
2 to <16 yr	14	1	2×10^{-5}	5	1.0×10^{-4}	7	1.4×10^{-4}	7	1.4×10^{-4}	20	4.0×10^{-4}	3	6.0×10^{-5}
16 to 70 yr	55	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}
Total lifetime risk			1.0×10^{-4}		2.2×10^{-4}		3.1×10^{-4}		4.7×10^{-4}		1.6×10^{-3}		1.7×10^{-4}

[1] Risk accrued in age window = potency × ASF × exposure rate × (years exposed/70 years)

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[2] ASF derived using equal weighting of studies within a chemical (i.e., Method 1 in main text)

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Similar, *albeit* more limited conclusions regarding sensitivity of the young to carcinogens were reached by the U.S. Environmental Protection Agency (U.S. EPA, 2005), in its *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*. Specifically, the U.S. EPA (2005) concluded that there is evidence of differential susceptibility for mutagenic carcinogens and recommended adjustments to the adult cancer slope factor and to estimate cancer risk from early life exposure. The U.S. EPA (2005) policy is to determine whether a chemical operates by a mutagenic mode of action, and for those that do, apply a ten-fold adjustment to the adult cancer slope factor for exposures occurring from birth up to two years of age, and a three-fold adjustment for such exposures occurring from 2 up to 16 years of age. The U.S. EPA (2005) does not adjust for increased susceptibility of the fetus to carcinogen exposures, or for the full lifetime ahead for cancer to manifest following early life exposures. It also does not apply any adjustments for non-mutagenic carcinogens, even though there is increasing appreciation of the importance of epigenetic and other non-mutagenic mechanisms in carcinogenesis, and recognition of epigenetic changes as early events in human carcinogenesis (Baylin, 2005).

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The U.S. EPA's factor of 10 for postnatal exposures falls just below the median estimate for the postnatal ASF (See Table 1). Thus, while it is consistent with the multi-lifestage exposure studies analysis presented here, it may result in underestimates of risk for a reasonable fraction of chemicals. The U.S. EPA's factor of three for juvenile exposures is generally consistent with the juvenile ASF derived from the multi-lifestage exposure studies, although it falls below the median estimate. It is acknowledged that there are few data available on which to base an estimate for the juvenile lifestage. A factor of three accounts for the long available time for cancer to manifest when exposure occurs in this period, but would not fully account for inherent differences in susceptibility to cancer, as is observed in breast tissue of pubescent girls exposed to radiation.

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The U.S. EPA and existing California practice does not estimate contributions from prenatal carcinogen exposure when estimating lifetime cancer risk. This is an implicit assumption in risk calculation that risk from prenatal exposure is zero. As shown in the multi-lifestage exposure studies analysis presented here, this assumption is inconsistent with the available evidence.

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Moreover, the analysis presented here suggests that a prenatal adjustment factor to the adult potency is needed; a factor of 10 falls roughly at the 70th percentile for the prenatal multi-lifestage exposure studies; the mean value is 21.

Table 1 shows how the application of the U.S. EPA's adjustment factors to calculate lifetime cancer risk compares with the use of the ASF values derived from the multi-lifestage exposure studies here. For example, the use of 70th percentile ASF values as adjustments for the prenatal, postnatal, and juvenile periods increases the lifetime cancer risk estimate almost two-fold above that estimated using the U.S. EPA's adjustment factors.

Concluding Remarks

OEHHA recognizes the limitations in the data and analyses presented, including limitations associated with the number and types of carcinogens with multi-lifestage exposure data; the non-homogeneous nature of the available multi-lifestage exposure studies; the focus on broadly defined lifestages, without attempting to describe changes in susceptibility that occur within those broadly defined lifestages; and the use for several studies of juvenile animals as the later life exposure group in cases where no adult exposure group was included. In addition, the assumption that the cancer hazard function increases with the third power of age may result in an underestimation of the true sensitivity of these early lifestages, if the true rate of increase with age is greater than that.

Still the analyses do provide some guidance on the extent risk may be over- or underestimated by current risk assessment approaches. The analyses support the application of weighting factors to address potential increased susceptibility to carcinogen exposures occurring prenatally and during the postnatal and juvenile lifestages.

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Background

Early-in-life susceptibility to carcinogens has long been recognized by the scientific community and clinicians as a public health concern. Numerous scientific publications and symposia have addressed this issue over the years (e.g., Toth, 1968; Rice, 1979; Napalkov *et al.*, 1989; NRC, 1990; 1993; 1994; Anderson *et al.*, 2000; Miller *et al.*, 2002; Birnbaum and Fenton, 2003; Ginsberg, 2003; Hattis *et al.*, 2004; 2005; Barton *et al.*, 2005). The scientific literature contains a number of human clinical findings and epidemiological studies of early life cancer susceptibility.

Table 2 provides examples of various human cases of early life cancer susceptibility. In the early 1960's, clear cell vaginal adenocarcinoma began appearing in teenagers and young women whose mothers took the synthetic estrogen diethylstilbestrol (DES) to avoid pregnancy loss (Herbst *et al*., 1971; Preston-Martin, 1989). Observations of marked differences in breast cancer risk in teenage compared to pre-pubescent girls treated for Hodgkin's disease with X-irradiation (Bhatia *et al.*, 1996) underscored the importance of considering life stage in assessing risks of cancer treatment and follow-up to it. The susceptibility of the fetus, infants, and children to thyroid carcinoma following exposure to radioactive iodine (Moysich *et al.*, 2002) and of children under 18 years of age to post-transplant lymphoma (Penn, 2000) has also been recognized.

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Table 2. Examples of Early-Life Cancer Susceptibility in Humans

Agent (reference)	Susceptible Group	Case
Diethylstilbestrol (DES) (Herbst *et al*., 1971; Preston-Martin, 1989)	Fetus	*In utero* exposure arising from administration of DES during pregnancy resulted in an increased risk of adenocarcinoma of the vagina and cervix in the daughters, but not in mothers taking the drug
X-Irradiation treatment for Hodgkins lymphoma (Bhatia *et al.*, 1996)	Girls with developing breast tissue (10-16 years old)	10-16 year old girls considerably much more likely to develop breast cancer than those under age 10 similarly treated. Risk of cancer by age 40: 35%
Radioactive iodine fallout from the 1986 Chernobyl accident (Moysich *et al.*, 2002)	Fetus/children	An increased risk of thyroid carcinoma was observed in children from Ukraine and Belarus exposed to radioactive iodine fallout. The greatest risk of thyroid carcinoma was observed in children aged five and under at the time of the accident.
Immunosuppressive drug treatment associated with organ allograft (Penn, 2000)	Children ages 18 years or less	Children are more prone to develop post-transplant lymphomas and lymphoproliferative disorders than adults (53% vs. 15%)

While there are many indications of increased human cancer susceptibility in early life, the magnitude of the impact has been difficult to gauge, and until recently risk assessment procedures have not in general addressed the issue. The California legislature in 2000 recognized the need for a systematic approach to develop scientifically based methods to address this concern so that in environmental decision making special sensitivities of the developing fetus and the young were taken into account. The legislature directed the Office of Environmental Health Hazard Assessment (OEHHA) to assess methodologies used in addressing early-in-life risk, compile animal data to evaluate those methods, and develop methods to adequately address carcinogenic exposures to the fetus, infants, and children (Children's Environmental Health Initiative (AB 2872, Shelly); California Health and Safety Code [HSC] section 901 (a) through (e)).

Here the results of OEHHA's quantitative analyses and synthesis of data from studies in animals exposed to carcinogens during different lifestages are presented. First the compilation of data on which the analysis relies is described. This is followed by a description of methods used to analyze the data and derive measures of early-life susceptibility. The analytical approach first evaluates differences in age sensitivity in terms of exposures in different lifestages for individuals similarly exposed and followed for similar periods of time – characterizing the inherent susceptibility of the young to the carcinogen. The second step of the analysis takes into account the longer period of time that carcinogen exposure to the fetus, infant, or child has to manifest as cancer, by taking into account time-of-dosing and assuming, in an approach consistent with that used by the National Toxicology Program (NTP) in analyzing tumor incidences in its chronic bioassays, that cancer risk increases with the third power of age. Adjustment factors that would potentially account for early life exposures are then described. These factors, referred to as age sensitivity factors (ASFs), address both inherent susceptibility of the young and the available time since exposure to develop cancer (Figure 1). The work of other bodies or researchers that have suggested or adopted methods to address early life exposure is then described in the context of the analyses and adjustment factors presented here. The document concludes by illustrating the impact of lifestage-specific ASFs on calculated lifetime cancer risk, assuming in this example that carcinogen exposure occurs at a constant rate across all lifestages, from conception through age 70.

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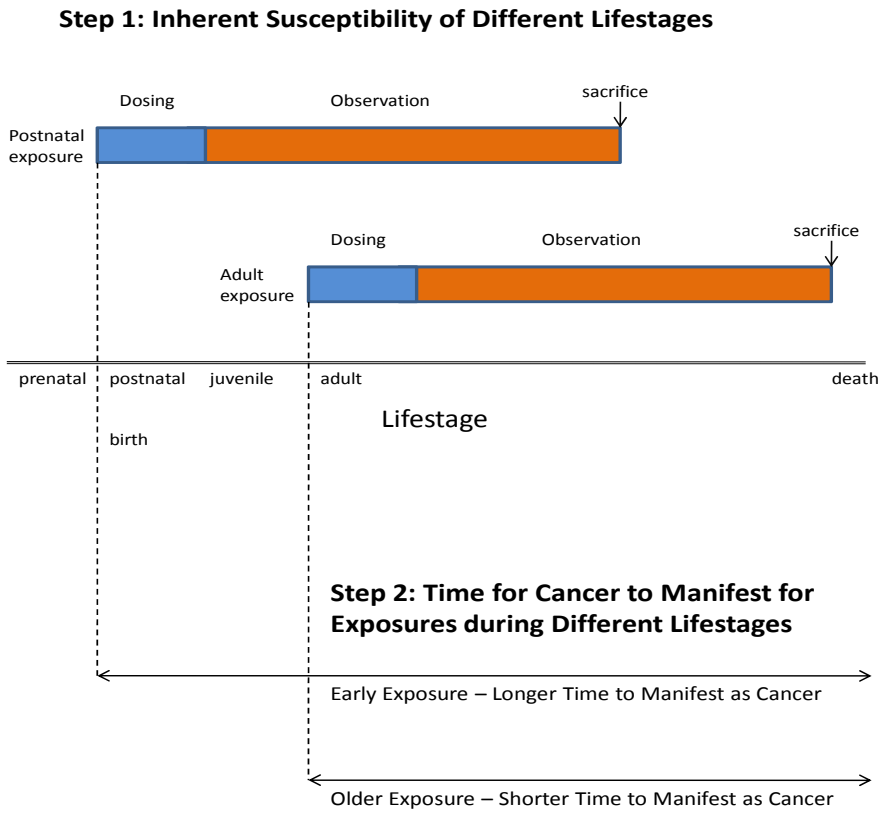
Figure 1. Issues addressed by the Age-Sensitivity Factor, or "ASF"



Animal Studies of Age Susceptibility

Lifestage Exposure Periods

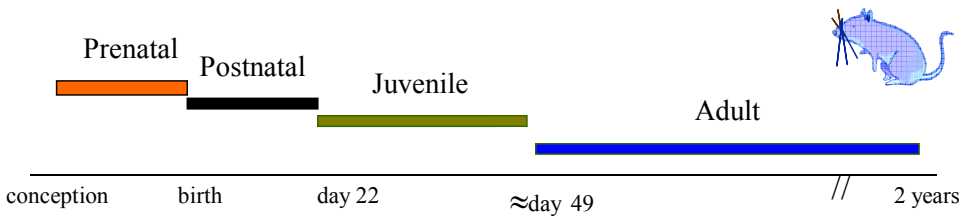
OEHHA has identified and compiled published animal cancer bioassays exploring age susceptibility issues. Two types of studies with early life carcinogen exposures were included in this effort. The first type are "multi-lifestage exposure studies." These studies have at least two

groups exposed during different lifestages. One dose group is exposed to a chemical only during one of the following lifestages:

- prenatal: from conception to birth
- postnatal: from birth to weaning
- juvenile: from weaning to sexual maturity (Figure 2).

The second dose group is exposed for some period of time at an older age, preferably during the adult lifestage, that is, after sexual maturity. This group served as the referent group. In some cases where there was no adult exposure group, animals exposed as juveniles served as the referent group. Studies or groups in which the exposure period for a given group spanned multiple life stages were not included in this effort. Multi-lifestage exposure studies are available for many chemicals, enabling the exploration of patterns in early-life susceptibility across chemicals.

Figure 2. Definition of Rodent Lifestage Adopted in the OEHHA Analyses



The second type are "single-lifestage exposure experiments." In these "single-lifestage exposure experiments" dose groups were exposed to a carcinogen only during a particular lifestage and, unlike the "multi-lifestage exposure studies," there was no requirement that the same study also include groups exposed during a different lifestage. Thus, single-lifestage exposure experiments were identified as being either prenatal, postnatal, juvenile, or adult exposure studies. OEHHA conducted "chemical-specific case studies" of early-life sensitivity for two specific carcinogens, ethyl-N-nitrosoamine (DEN) and N-ethyl-N-nitrosourea (ENU). For each of the two chemicals,

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there were many prenatal studies conducted that were compiled, analyzed, and grouped together. Postnatal studies from different publications were similarly compiled, analyzed and grouped together, as were juvenile studies. Adult studies were not available for either DEN or ENU, thus for both chemicals juvenile exposure studies served as the referent for prenatal studies, and for postnatal studies. These "chemical-specific case studies" were conducted to explore the feasibility of analyzing chemical-specific data on age susceptibility from single-lifestage exposure experiments.

There is little question regarding whether or not a certain bioassay group should be identified as receiving exposure for certain lifestages. For example, where exposure to dams ends at birth, offspring can be considered exposed during the prenatal period. The line between the juvenile and adult lifestages is less clear. Assumptions had to therefore be made to categorize exposures used in the bioassays into the lifestages named above. These assumptions were based on standard reference documents and consultation with developmental biologists and toxicologists. Table 3 gives the ages assumed in establishing the postnatal, juvenile, and adult lifestages for the species included in the compiled studies with early life exposure.

Table 3. Definition of Lifestages by Species[1].

Species	Postnatal: Birth to Weaning	Juvenile: Weaning to sexual maturity	Adult: Sexual maturity/breeding age
Rat – male	Day 1-21	Day 22-76	≥ Day 77 (10 wks)
Rat – female	Day 1-21	Day 22-55	≥ Day 56 (8 wks)
Mouse	Day 1-21	Day 22-48	≥ Day 49 (7 wks)
Hamster	Day 1-21	Day 22-48	≥ Day 49 (7 wks)
Gerbil	Day 1-28	Day 29-55	≥ Day 56 (8 wks)

[1]The prenatal lifestage is defined as the period from conception to birth for all species.
References: Merck, 1998; Harder *et al.* 1993; Fox *et al.*, 1995; Harkness and Wagner, 1995; Charles River, 1999.

Typical cancer bioassays such as those conducted in rats and mice by NTP involve exposing animals starting at six to eight weeks of age, which is the time at which these animals reach sexual maturity (late teenagers relative to humans). The experiments are run for two years, ending when the animal is in late middle age. Thus, early and very late life exposures are not

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¶
A second type of early life exposure study is used to examine age susceptibility for individual chemicals in depth. These studies support the "chemical-specific case study" of these individual carcinogens. For the case studies, there is at least one group of animals in a bioassay dosed during one of the four exposure windows named above, and there are multiple such bioassays available for each early life exposure window evaluated (e.g., prenatal, postnatal, juvenile). In the current report we present results for two case studies: diethylnitrosamine (DEN) and ethylnitrosourea (ENU). ¶

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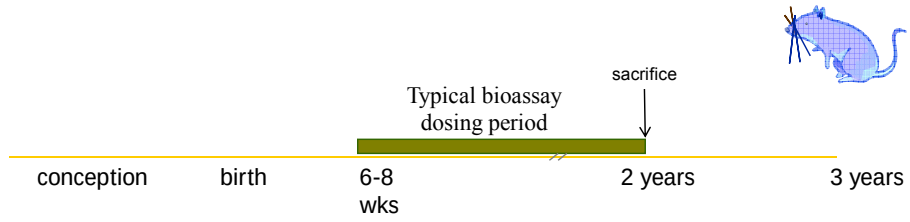
included in the typical rodent bioassay (see Figure 3). Thus OEHHA focused on finding studies that evaluated early in life exposures

Figure 3. Dosing Period for Typical Rodent Bioassays



Criteria for Study Inclusion

Bioassays examining responses in particular lifestages were for the most part designed by different researchers to explore issues related to age susceptibility of carcinogens. The research community did not for the most part standardize protocols for these studies. There is therefore a great deal of variation across studies in terms of dose selection, duration of exposure, number of animals, and length of study duration. To be included in the compilation of studies with early life exposure, a study or an experimental group in a study had to meet minimum requirements.

The criteria for study inclusion are as follows:

- Treated groups were exposed to a single chemical carcinogen or a single carcinogenic chemical mixture.
- Study groups were not compromised by severe treatment-related non-cancer toxicity.
- Overall the duration of exposure period plus observation period exceeded 40 weeks, unless animals died of tumor.
- For included dose groups, the study must report age at dosing, age at sacrifice, and site-specific tumor incidence.

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- Each lifestage exposure treatment group has an appropriate concurrent control group, or, for rare tumors only, an appropriate historical control.
- The studies were on mammals.
- Each treatment and control group consists of at least ten animals, unless the conduct and design of the study was well done in all other aspects (e.g., the length of the study was sufficiently long to observe treatment-related tumors) and tumor incidence was high in treated groups and very low in controls.
- Site specific tumor data were reported, and not only total number of tumor bearing animals.
- The test compound was administered in the diet, water, via gavage, or by intraperitoneal (i.p.), intravenous (i.v.), or subcutaneous (s.c.) injection. For dermal and subcutaneous injection studies, distal tumor findings are utilized (for dermal, other than skin tumors; for injection, non-injection site tumors).
- While studies designed to histopathologically examine tumors at multiple sites were preferred, studies that examined only a select set of organ/tissue sites were not excluded if the sites examined were known with confidence to be the only target tissues for the chemical and age exposure window in question in that particular strain of animal.

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Data Sources

Different approaches were taken to identify animal cancer studies that included groups of animals exposed during early lifestages. First, MEDLINE and TOXLINE (National Library of Medicine) databases were searched using combinations of various key words for cancer (e.g., tumor(s), neoplasm(s), cancer, neoplasia, cancerous, neoplasms-chemically induced) and for early-life exposure (e.g., age, age-at-exposure, development (al), prenatal, *in utero*, gestation (al), postnatal, neonatal, juvenile, weaning, weanling, adolescent, adolescence, young). Second, the extensive compilation of bioassays in the *Survey of Compounds which have been Tested for Carcinogenic Activity*, was reviewed. This survey, formerly maintained by the National Cancer Institute as Public Health Service Publication Number 149, or PHS 149, is now available from a private source electronically as CancerChem, 2000. Third, from bibliographies from relevant published papers additional studies were identified. Finally the Single Dose Database developed

by Calabrese and Blain (1999) was obtained and utilized to identify additional publications that appeared to contain potentially useful data. All of these publications were evaluated to determine if the study dosed separate groups of animals early in life and at or near adulthood. A total of 145 publications, providing data on 84 chemicals, were identified as meeting the criteria for study inclusion. A subset of these met the criteria for inclusion in the multi-lifestage exposure analysis.

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An Experiment

Here we define an experiment as a study component consisting of a control group as well as a treated group(s) exposed during the same lifestage (i.e., prenatal, postnatal, juvenile or adult), and using the same experimental protocol (e.g., route of exposure, strain, species, laboratory). One publication may be a report for multiple experiments.

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Multi-Lifestage Exposure Studies

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Thirty-six of the 145 publications containing studies that met the selection criteria described above reported multi-lifestage exposure studies (Figure 2 and Table 3), that is, they included at least one group dosed solely in a defined early lifestage (prenatal, postnatal or juvenile), a control group and a comparison group of animals exposed only as adults (preferred) or in some cases, juveniles. Thus a multi-lifestage exposure study contains multiple experiments – at least one experiment with exposure in a prenatal, postnatal or juvenile lifestage, and another experiment with exposure in an older group, preferably adults. The publications on the multi-lifestage exposure studies are listed in Table 4.

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As indicated in Table 4, sixteen of the 36 multi-lifestage exposure publications included groups of animals dosed only during the prenatal period, providing data on 14 chemicals. Twenty-five of the multi-lifestage exposure publications included groups of animals dosed only during the postnatal period, providing data on 18 chemicals. Five of the multi-lifestage exposure publications included groups of animals dosed only during the juvenile period, as well as groups of animals dosed only during the adult period, and provided data on five chemicals. Experimental animal species employed in these studies included rats, mice, gerbils, and hamsters.

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Table 4. Multi-Lifestage Exposure Studies

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Chemical, CAS Number	Species	Exposure Lifestages[1]				Publication
		Pr	Po	Ju	Ad	
Benzidine, 92-87-5	Mouse		√	√		Vesselinovitch *et al.*, 1975b
		√	√	√		Vesselinovitch *et al.*, 1979a
Benzo[a]pyrene, 50-33-9	Mouse		√	√		Vesselinovitch *et al.*, 1975a
			√	√		Truhaut *et al.*, 1966
1,1-Bis(*p*-chlorophenol)-2,2,2-trichloroethane (DDT), 50-29-3	Mouse		√	√		Vesselinovitch *et al.*, 1979a
Butylnitrosourea, 869-01-2	Rat	√	√	√		Zeller *et al.*, 1978
Dibutylnitrosamine, 924-16-3	Mouse		√		√	Wood *et al.*, 1970
Diethylnitrosamine (DEN), 55-18-5	Mouse		√	√		Rao and Vesselinovitch, 1973
	Mouse[a]		√	√		Vesselinovitch *et al.*, 1984
	Hamster	√			√	Mohr *et al.*, 1975[e]
		√			√	Mohr *et al.*, 1995
Diethylstilbesterol (DES), 56-53-1	Mouse	√			√	Turusov *et al.*, 1992
7,12-Dimethylbenz[a]anthracene (DMBA), 57-97-6	Rat		√	√	√	Meranze *et al.*, 1969
	Mouse		√		√	Walters, 1966
1,2-Dimethylhydrazine, 540-73-8	Rat		√	√		Martin *et al.*, 1974
Dimethylnitrosamine (DMN), 62-75-9	Hamster	√			√	Althoff *et al.*, 1977
	Rat			√	√	Noronha and Goodall, 1984
Di-*n*-propylnitrosamine (DPN), 621-64-7	Hamster	√			√	Althoff *et al.*, 1977
		√			√	Althoff and Grandjean, 1979
1-Ethylnitrosobiuret, 32976-88-8	Rat	√	√		√	Druckrey and Landschutz, 1971
Ethylnitrosourea (ENU), 759-73-9	Gerbil		√	√		Naito *et al.*, 1985
	Rat		√	√		Bosch, 1977
		√	√	√		Naito *et al.*, 1981
		√			√	Tomatis *et al.*, 1977
	Mouse[a]		√	√		Vesselinovitch *et al.*, 1974
2-Hydroxypropylnitrosamine, 39603-53-7	Hamster	√			√	Althoff and Grandjean, 1979
3-Hydroxyxanthine, 13279-29-3	Rat		√	√	√	Anderson *et al.*, 1978

Table 4. Multi-Lifestage Exposure Studies (continued)

Chemical, CAS Number	Species	Exposure Lifestages[1]				Publication
		Pr	Po	Ju	Ad	
3-Methylcholanthrene (3-MC), 56-49-5	Mouse	√			√	Tomatis *et al.*, 1971
			√		√	Klein, 1959
		√			√	Turusov *et al.*, 1973
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 64091-91-4	Mouse	√			√	Anderson *et al.*, 1989
Methylnitrosourea (MNU), 684-93-5	Rat			√[c]	√[d]	Grubbs *et al.*, 1983
	Mouse		√	√		Terracini and Testa, 1970
			√		√	Terracini *et al.*, 1976
β-Propiolactone, 57-57-8	Mouse		√		√	Chernozemski and Warwick, 1970
Safrole, 94-59-7	Mouse	√	√	√		Vesselinovitch *et al.*, 1979a
		√	√	√		Vesselinovitch *et al.*, 1979b
Tetrachlorodibenzodioxin (TCDD), 1746-01-6	Mouse		√[b]	√		Della Porta *et al.*, 1987
Urethane, 51-79-6	Rat	√	√	√	√[e]	Choudari Kommineni *et al.*, 1970
Vinyl chloride, 75-01-4	Rat	√	√		√	Maltoni *et al.*, 1981

[1] Abbreviations: prenatal, Pr; postnatal, Po; Juvenile, Ju; adult, Ad.
[a] Conducted in two strains of mice.
[b] Postnatal dosing extended slightly into the juvenile period.
[c] Dosing initiated toward the end of juvenile period in female rats, from day 50 to 57.
[d] There were two adult female rat exposure groups, one exposed from day 80 to 87, and the other from day 140-147.
[e] Dosing initiated in later part of the juvenile period, from day 46 to 61.

Chemical-Specific Case Studies Data: DEN and ENU

DEN and ENU are two well-studied model carcinogens, and their modes of carcinogenic action and pharmacokinetic behaviors are relatively well understood. They both are genotoxic, and form DNA adducts. DEN requires metabolic activation, while ENU does not. They both cross the placenta. There are numerous experiments on DEN and ENU included in the compilation of studies with early life exposure. For these reasons, these chemicals were selected as case studies. Cancer potencies, defined below, were derived using the data from single-lifestage exposure studies. Only data in the mouse were used, as this was the species in which the largest numbers of early life exposure experiments were conducted on DEN and ENU.

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DEN. Ten mouse publications on DEN were identified (See Table 5). Among these publications, three included studies of mice exposed during the prenatal lifestage, seven included studies of mice exposed during the postnatal lifestage, and two included studies of mice exposed during the juvenile lifestage. These publications yielded a total of eight prenatal datasets, 18 postnatal datasets, and five juvenile datasets. No "adult only" exposure studies were identified in mice for DEN. Thus the juvenile lifestage exposure studies were used as the older age at exposure comparison group. If the juvenile lifestage is more susceptible to DEN exposures than the adult, then the use of these juvenile exposure studies as the comparison group will result in an underestimate of the early life susceptibility associated with prenatal and postnatal exposure to DEN.

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Table 5. DEN and ENU Mouse Single-Lifestage Exposure Experiments

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Chemical, CAS Number	Exposure Lifestages[1]				Publication
	Pr	Po	Ju	Ad	
Diethylnitrosamine (DEN), 55-18-5	√				Anderson *et al.* (1989)
		√			Boberg *et al.* (1983)
		√			Drinkwater and Ginsler (1986)
		√			Lai *et al.* (1985)
	√				Mohr and Althoff (1965)
		√	√		Rao and Vesselinovitch (1973)
		√			Turusov *et al.* (1973)
		√	√		Vesselinovitch *et al.* (1984)
		√			Vesselinovitch (1980)
	√				Vesselinovitch (1983)
Ethylnitrosourea (ENU), 759-73-9		√			Anderson *et al.* (1989)
	√				Diwan *et al.* (1974)
		√			Drinkwater and Ginsler (1986)
	√				Kaufman (1976)
		√			Naito *et al.* (1982)
		√			Pereira *et al.* (1985)
		√			Schmahl (1988)
		√			Searle and Jones (1976)
			√		Vesselinovitch *et al.* (1973)
		√	√		Vesselinovitch *et al.* (1974)
	√				Vesselinovitch *et al.* (1977)
	√	√	√		Vesselinovitch (1983)
	√				Wiggenhauser and Schmahl (1987)

ENU. Thirteen mouse publications on ENU were included in the compilation of studies with early life exposure (See Table 5). Of these, five had studies on mice exposed during the prenatal period, eight during the postnatal period, and three during the juvenile period. These publications yielded a total of 30 prenatal datasets, 27 postnatal datasets, and eight juvenile datasets. As with DEN, no "adult only" exposure studies were identified, and if the juvenile lifestage is more susceptible to ENU exposures than the adult, then the use of these juvenile exposure studies as the comparison group will result in an underestimate of the early life susceptibility associated with prenatal and postnatal exposure to ENU.

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Methods

This section describes the methods used to analyze and compare the carcinogenic activities of compounds in different lifestages. First a measure of carcinogenic activity, the cancer potency, is defined. Methods for deriving it from animal studies are then described. The ratio of cancer potency derived from an early lifestage exposure experiment to that derived from an experiment conducted in adult animals, referred to as a lifestage potency (LP) ratio, was calculated for each multi-lifestage exposure study. The LP ratio characterizes the inherent susceptibility of early lifestages to carcinogen exposure, by comparing potencies for individuals followed for similar periods of time and similarly exposed, but exposed during different lifestages. The longer period of time that exposed young have to develop tumors is addressed by taking into account time-of-dosing, and assuming that cancer risk increases by the third power of age. This was done by multiplying the LP ratio by a time-of-dosing factor, to yield the ASF, thus taking into account the overall sensitivity associated with early-life exposure. Cancer potencies, LP ratios, and ASFs are estimated from data and not measured precisely. To describe this uncertainty, these measures are described by probability distributions. Methods for the derivation of these distributions are also explained below.

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Deleted: ," measures the carcinogenic activity in an early lifestage exposure window compared to adult exposure. The ASF is defined simply as the ratio of the cancer potency in an early age window to the cancer potency in the adult age window.
Deleted: For the multi-window studies, ASFs are derived for each experiment, and also for the different chemicals. For the DEN and ENU case studies, cancer potency is derived for each experiment. These in turn are used to derive distributions of cancer potency within a given life stage exposure window. There are no "adult only" exposure experiments for DEN and ENU in mice. The ratios for prenatal to juvenile potencies and postnatal to juvenile potencies are instead derived. Statistical methods employed for this are explained below.
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Cancer Potency

Mathematic Model. Cancer potency estimates were derived by applying a linearized multistage (LMS) model to cancer dose-response data from studies in experimental animals. This model

was chosen because of widespread use in risk assessment, and its flexibility in being able to fit many different data sets needed to evaluate the effect of lifestage-at-exposure on cancer potency. Assuming dose-response is linear at low doses, the LMS model provides a mechanism of bounding the quantitative estimates of low-dose risk from exposures to carcinogenic agents (Crump *et al.*, 1976; Peto, 1978). The LMS model may be described by the following equation

$$p(d) = 1 - e^{-\sum_{i=0}^{k-1} q_i d^i}, \quad q_i \geq 0, \qquad (1)$$

where p(d) represents the lifetime probability of cancer at a lifetime dose rate, d, and q_i are model parameters that were estimated via maximum likelihood methods, as described below. At low doses the above equation reduces to:

$$p(d) = 1 - e^{-(q_0 + q_1 d)}$$

When q_0 is small this reduces to the simple linear relationship:

$$p(d) = q_0 + q_1 d.$$

where the probability of cancer is represented in the unexposed by intercept q_0 and in the exposed increases linearly with dose d. Here, cancer potency is defined as the parameter q_1: At low doses, it describes quantitatively the extent that cancer risk increases with an incremental increase in dose.

Dose Metric. The work here is to compare cancer potencies from experiments utilizing the same protocols but for the lifestage in which dosing occurred. The dose metric adopted for this work is the cumulative dose normalized by bodyweight:

$$d = \sum_i^t d_i$$

d_i, the dose given on i^{th} day of the experiment, is expressed in units milligram amount administered per kilogram animal bodyweight (mg/kg-bw). This results in potencies that are comparable in terms of the initial internal concentration after administration of the compound, and the overall exposure during the lifestage. The cancer potency q_1 is expressed as the increase in risk with increasing cumulative dose, in units mg/kg-bw.

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Experiments did not always report dose administered in units mg/kg-bw. When dose was reported as a concentration administered in diet or water, it was converted to mg/kg-bw based on the amount of food or water consumed, the concentration in the media and the body weight of the animal on the day of dosing. When dose was reported as bulk quantity applied (e.g., mg amount), it was converted to mg/kg-bw by dividing by the body weight of the animal on the day of dosing.

If the body weight on the day(s) of dosing was not reported in the publication, the default body weight was used. The default body weights of rats and mice were modeled from normative data on common strains of mice (BALB/cANCr, AKR/LwCr, and C57Bl/6Cr) surveyed by Poiley (1972) and rats (Fischer 344 and Sprague-Dawley) surveyed by Poiley (1972) and Cameron *et al.* (1985) using constrained linear regression and the statistical package STATA (Stata Corp, College Station, Texas). The model takes the form:

$$\text{BodyWeight}_{age} = \beta_0 + \beta_1 (\text{day-1}) + \beta_2 (\text{day-1})^2 + \beta_3 (\text{day-1})^3 + \beta_4 (\text{day-1})^4 ,$$

where β_0 was defined as the measured average body weight on day 1 of life (i.e., redefining day 1 as 'day 0' or the origin). The variable day is the day of life, and parameters, β_1, β_2, β_3, β_4 are estimated. Fitted values for each day of life from birth through six months of age (i.e., day 168) for male and female mice (applied to all strains) and male and female rats (separate body weight tables are given for Sprague-Dawley rats and all other strains) are provided in Appendix A.

Procedure to Estimate Cancer Potency

Model parameters were estimated using maximum likelihood methods, using a forward selection process. The forward selection process commences with the data being fit to a two-parameter LMS model. If the goodness-of-fit test indicates an adequate fit (at the $p = 0.05$ level) then the two-parameter LMS model is used to compute the cancer potency. If the two-parameter model does not satisfactorily fit the data, a three-parameter model is fit. This model is then assessed via a goodness-of-fit test. The process of adding an additional parameter and assessing model fit continues until the goodness-of-fit statistic is no longer statistically significant.

In some cases the dose response data are not consistent with an upward curving dose response relationship, such that tumor incidence can initially increase with dose and then remain steady or decrease as doses are further increased. This can occur from competing causes of mortality such as cancers at sites other than the one being analyzed, and other causes of death. It can also result from pharmacokinetics for example when a chemical requires activation for carcinogenic activity, and the activation pathway saturates as dose is increased. Following the inclusion criteria described above, when mortality from noncancer toxicity is high, the study is not suitable for inclusion in the data base. There are a few datasets included in these analyses where, despite meeting the study inclusion criteria, the LMS model does not fit the data well. For these datasets, a procedure laid out in Anderson *et al.* (1983) is used to remove high dose groups. Working down from the highest dose group, dose groups are removed, the model fitted, until there is an adequate fit of the model to the data (goodness-of-fit, $p > 0.05$).

The analysis begins by focusing on experiments conducted with exposures in a given lifestage and deriving cancer potency estimates for each experiment conducted with groups exposed during that lifestage.

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The method of maximum likelihood is implemented to obtain the model parameter estimates for each experiment. Here the parameter of greater interest is the potency, q_1, the slope term in equation (1). The idea behind maximum likelihood parameter estimation is to determine the parameters that maximize the probability (likelihood) of observing the sample data. For each animal, the probability of cancer is given by equation (1). Assuming each animal exposed to dose d_i within a given lifestage has the same chance of developing cancer at a specific site (or arising from a specific cell type), the probability of observing r_i animals with that cancer out of n_i animals total may be described by the following binomial distribution,

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$$\binom{n_i}{r_i}[p(d_i)]^{r_i}[1-p(d_i)]^{(n_i-r_i)}. \qquad (2)$$

For a given experiment, there are different dose groups, that is d_i is the same for each animal within the dose group, but differs across the dose groups. The likelihood is constructed by assuming that animals across the dose groups are independent, and the likelihood is the product of the term (2) above across the k dose groups or categories, i.e.,

$$L([q_0,q_1,\ldots,q_{k-1}]) = \prod_{i=0}^{k-1}\binom{n_i}{r_i}[p(d_i)]^{r_i}[1-p(d_i)]^{(n_i-r_i)}. \quad (3)$$

The support function, also referred to as the log-likelihood, is defined as the natural logarithm of the likelihood function (3), disregarding constants, i.e.

$$S([q_0,q_1,\ldots,q_{k-1}]) = \ln L([q_0,q_1,\ldots,q_{k-1}])$$
$$= \sum_{i=0}^{k-1} r_i \ln[p(d_i)] + (n_i - r_i)\ln[1-p(d_i)]. \quad (4)$$

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The values of q_0, q_1, . . . , q_{k-1} that maximize equation (4) are the maximum likelihood estimates. Profile-likelihood methods are used to trace the likelihood to determine the 0.5% through the 99.5% (in increments of 0.5%) confidence bounds of the linear slope parameter of the model, q_1. This is done to describe the uncertainty in the estimates of this parameter, as well as the confidence we may have that the parameter falls below some upper bound value. Determining the confidence percentiles of the slope parameter q_1 provides a discretized distribution of this parameter.

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The above procedure is performed for each treatment related tumor site or type in the experiment, that is for each site or type for which a treatment-related increase in tumors has occurred (i.e., a statistically significant increase in tumor response in the exposed compared to the treatment group [$p \leq 0.05$], or a biologically significant finding of rare tumor). For studies in which a carcinogen causes tumors at multiple sites or of multiple types, a combined "multisite" potency distribution is estimated from the site/type-specific potency distributions. A combined distribution of cancer potency is created by statistically summing across the site/type specific potency distributions for each treatment-related tumor site/type in the experiment, using a Monte Carlo procedure with 100,000 iterations per experiment. In performing this analysis the cancers at the different sites/types are assumed to be independent. The result of this procedure is an estimate of potency for the total treatment caused cancer burden observed in the experiment (Figure 4).

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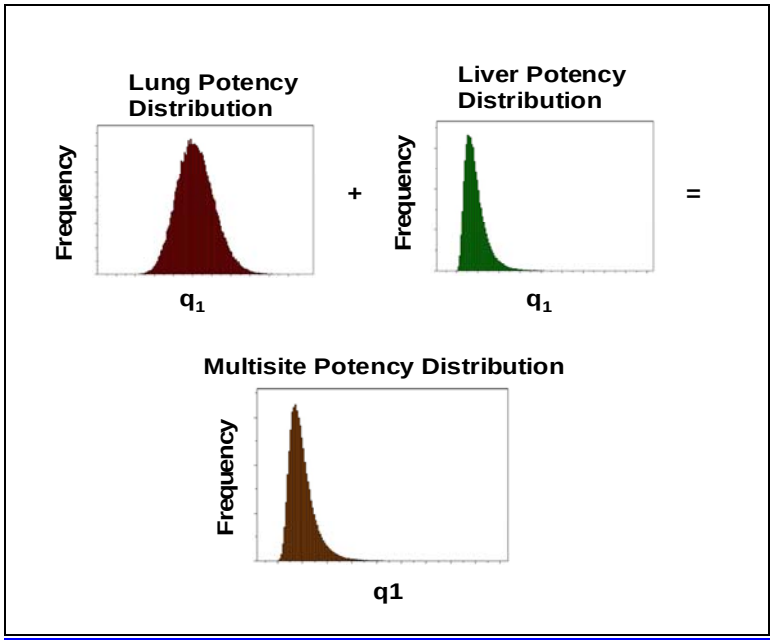
Figure 4. Addition of potency distributions for multi-site cancer potency derivations



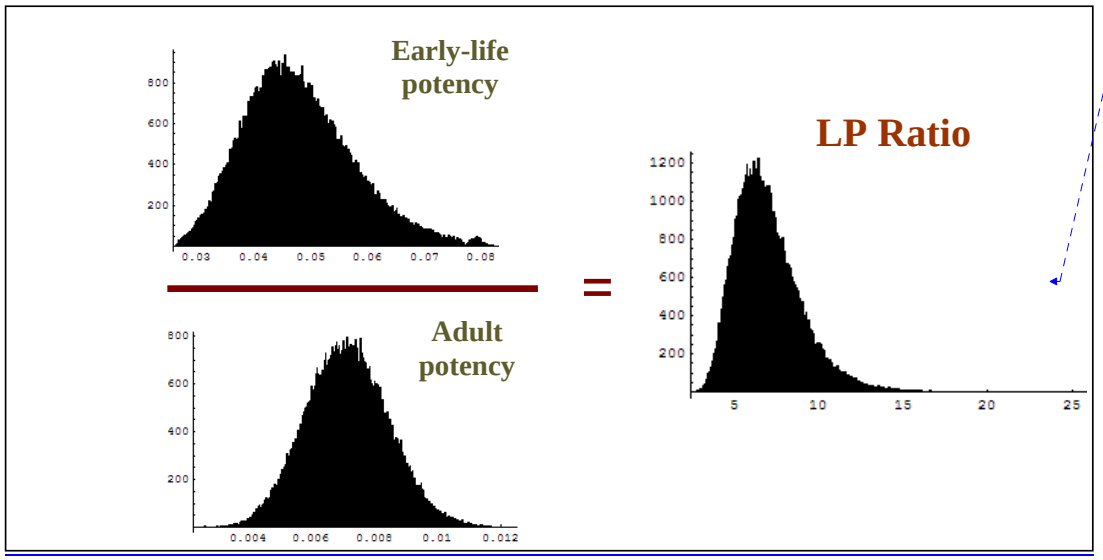
In a given experiment, not all groups were observed for the same length of time. Therefore in computing potency for a given exposure lifestage within a study, all observation periods were normalized to the same time length (t_{obs}), typically the observation period for the control animals. For the purpose of this calculation the observation period is defined as the time between the age at the initiation of dosing (t_d) and the age the animals were killed (t_m). Following the NTP (Bailer and Portier, 1988), cancer was assumed to increase with the third power of age and an adjustment $(t_m - t_d)^3 / t_{obs}^3$ was applied to each group. In cases where all groups were observed for the same period, adjustment was not necessary. For the single-lifestage exposure experiments analyzed in the chemical-specific case studies, all potency distributions were adjusted to a two year observation period.

Use of ASFs to Address Early-Age Sensitivity in Estimating Cancer Risk

Inherent Sensitivity of Lifestages – Lifestage Potency Ratios

Cancer potency is derived for each experiment, which again consists of groups of animals (e.g., all dosed within the same defined lifestage (i.e., prenatal, postnatal, juvenile, or adult), and following a similar experimental protocol but for dose level. In some cases different groups of animals were dosed at the same level (e.g., on a mg/kg-bw basis) on different days within the same lifestage (e.g., postnatal day 1 vs. postnatal day 15). If tumor incidences were not statistically significantly different (at the p = 0.05 level) between the groups dosed on different days within the same lifestage, the incidence data from the groups were combined. If a statistically significant difference was observed, then each of the groups was treated as a separate experiment. For each lifestage, a potency distribution is obtained for each experiment conducted. The cancer potency from "early life" exposure was compared to that from "later life" exposure. This comparison is facilitated by taking the quotient of the cancer potency distribution for those animals exposed in early life and those animals exposed in later life. This ratio distribution for multi-lifestage exposure studies is termed the lifestage potency (LP) ratio distribution (Figure 5).

Figure 5. Lifestage Potency Ratio (LP) Ratio Distribution



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For example the prenatal LP ratio is given by:

$$\textit{Prenatal LP ratio} = q_{1prenatal} \div q_{1\ adult}$$

The dividend is the cancer potency distribution for the prenatal exposure period $q_{1prenatal}$ and the divisor is the cancer potency distribution for the adult exposure period $q_{1\ adult}$ (Figure 5). Thus, the quotient distribution represents the spectrum of cancer induction sensitivity in an early-life stage relative to adults (or, in some instances juveniles when adult data are not available).

Of particular importance is the location of the LP ratio distribution in relation to the reference value of 1.0. An LP ratio distribution that primarily lies above the value of 1.0 indicates early life exposures to a carcinogen result in a stronger tumor response relative to adult exposure. Conversely, an LP ratio distribution that mainly lies below the value of 1.0 indicates early life exposure to a carcinogen results in a weaker tumor response relative to adult exposure.

Effect of longer time period for cancer to manifest

The LP ratios described above characterize the inherent susceptibility of the young compared to older animals to the carcinogen. The exposures were for individuals similarly exposed and followed for similar periods of time. Age-specific adjustments to the cancer potency must also take into account the longer period of time that carcinogen exposure to the young has to manifest as cancer. These LP ratios do not address this. Empirical data from studies of both humans and animals demonstrate that, for many cancers, cancer risk increases with age, or time since first exposure. While some cancers have been seen to increase by as much as the sixth power of age, a general approach taken for example by the NTP in analyzing tumor incidences in its chronic bioassays is to assume that cancer risk increases by the third power of age (the poly-3 correction) (Bailer and Portier, 1988).

The approach taken by the NTP and used here is based on the Armitage and Doll (1954) mathematical description of carcinogenesis. This approach has been applied in various contexts to consider the impact of dosing and observation at different ages (see e.g., Murdoch *et al.*, 1992; Crouch, 1983; and Crump and Howe, 1984). The model assumes that cancer derives from a

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single cell after it has undergone a series of transformations. While there have been numerous scientific developments advancing the understanding of carcinogenesis since Doll and Armitage first published their model, the model nonetheless provides a good statistical description of age dependent observations of cancer development. Thus, this is the context in which the model is applied here.

Assumptions are required for the application of the Doll-Armitage model regarding: 1) the mathematical relationship between applied dose and the probability that a "stage transition" has occurred, 2) the stage affected by the carcinogen and 3) the number of "stages." For the particular forms used to fit the tumor data in this report, a linear relationship is assumed between dose and cell transformation, and the carcinogen is assumed to affect an early stage of the cancer process.

If the probability per unit time of the stage transformation depends linearly on dose rate ($d(t)$), and the carcinogen only affects a single "stage," the probability of tumor by time T_e becomes

$$P(T_e) = 1 - \exp[-(A + BD)] \qquad (5)$$

with

$$D = \frac{1}{T^m \cdot \beta(m - j + 1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt \qquad (6)$$

where T_e is the time to observation, and β is Euler's beta function (see Crouch, 1983; Murdoch *et al.*, 1992). Here the adjustment is developed for analyses in rodents, so the default lifetime of the test animal is assumed. Following Anderson *et al.* (1983) this is two years for rats and mice. The integer m (the number of "stages") specifies the rate of increase in incidence with time and j is the "stage" affected by the carcinogen. To adjust for less than lifetime experiments in estimating cancer potency, the hazard function is assumed to increase with the third power of age, corresponding to a value for m of 3.0. The chemicals here demonstrably act at an early stage, and it is assumed therefore $j = 1$. the solution to Equation 6 describing the constant daily dose (D) equivalent to a daily dose d given over a time interval from a to b becomes, for $j = 1$ and $m = 3$:

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$$D = d \cdot \left[\frac{(T_e - a)^3 - (T_e - b)^3}{T^3} \right]. \qquad (7)$$

The intervals used to calculate the adjustment factors for each of the three early lifestages are: the day of birth for the prenatal period and from birth to age 21 days for the postnatal period. The juvenile and adult multi-lifestage exposure studies are in the rat; the interval used for the adjustment is age 22 to 65 days, with 65 days being the midpoint between sexual maturity for the female and male rats. Inserting these intervals into Equation 7, and comparing the result with the average lifetime daily dose associated with dosing in that age interval provides the adjustment factor. The time-of-dosing factors for the prenatal, postnatal and juvenile windows are 3, 2.9 and 2.7, respectively. Thus, ASFs were developed for each experiment, by first calculating the LP ratio to address inherent susceptibility of early lifestages relative to adults, and then accounting for the effect of years available to manifest a tumor following carcinogen exposure by multiplying the LP ratio by the appropriate time-of-dosing factor (see Figure 1).

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Deriving LP Ratios and ASFs for Multi-Lifestage Exposure Studies

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For each early lifestage, LP ratios are derived for each study with experiments for which a chemical was administered during that exposure period. These different chemical carcinogens act by a variety of mechanisms, and with varying pharmacokinetic properties in different lifestages. In addition, any given chemical can have multiple studies, sometimes in different species, strains and gender. LP ratios differ for the different studies performed on the same chemical and for the different chemicals. Combining these LP ratio distributions across all chemicals within a specific early lifestage results in a description of the magnitude and variability of age-at-exposure effects for the studies analyzed on these different chemicals. This provides a means by which the susceptibility of that lifestage to carcinogen exposure relative to the adult may be characterized for the data analyzed.

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A single “LP ratio mixture distribution” for each early lifestage is derived via Monte Carlo re-sampling methods across all of the chemicals representing a given lifestage. This LP ratio mixture distribution for a particular lifestage describes the variability in the LP ratio across these chemicals, and the uncertainty in the LP ratio.

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LP ratio mixture distributions for a given early lifestage were developed by (1) obtaining a single LP ratio distribution for each chemical (when a chemical is represented by more than one study) and (2) equally sampling across all chemicals. When a chemical is represented by more than one study, the LP ratio distributions from all studies of that chemical were combined to achieve one distribution to represent that chemical's LP ratio. This was done by equally sampling from each LP ratio distribution for each study via Monte Carlo methods. Once each chemical is represented by a single LP ratio distribution, the LP ratio mixture distribution for each early lifestage (e.g., prenatal) is obtained by equally sampling across all of the chemicals via Monte Carlo methods.

Two sensitivity analyses were also conducted, employing two alternative sampling methods to obtain a single LP ratio distribution to represent each chemical, for cases where a chemical was represented by more than one study. In the first sensitivity analysis, for each chemical with multiple studies, each study's LP ratio distribution is sampled based upon an inverse-variance weighting scheme. The variance is calculated for the logarithm of the LP ratio, and the likelihood that an LP ratio distribution is sampled is proportional to the inverse of this variance. In the second sensitivity analysis, the study with the largest median LP ratio is used to represent the chemical, that is, the LP ratio distribution for that study is used to represent the LP ratio for the chemical.

The LP ratio distribution for each chemical is used to derive the LP ratio mixture distribution for the group of chemicals. For each chemical, an LP ratio value is randomly chosen, according to its LP ratio distribution. This process proceeds for each of the chemicals in the group and is replicated 1,000,000 times to derive an LP ratio mixture distribution for the group. Mixture distributions are derived for each early lifestage.

Chemical-Specific Case Studies

The DEN and ENU case studies were limited to studies in mice. Mouse experiments for the adult lifestage are not available for either of these chemicals. Thus, for these chemicals prenatal and postnatal cancer potencies are compared to juvenile cancer potencies.

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Method 1 − Each of the ASF distributions are equally likely to be selected.

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Method 2 − Each of the ASF distributions is sampled based upon an inverse-variance weighting scheme. In this case, the variance is calculated for the distribution of the logarithm of the ASF, Var[logASF]. The likelihood that an ASF is sampled is proportional to 1/Var(log[ASF]). The variance of the logarithm of q_1 is used because potencies tend to differ by factors rather than in a linear fashion.¶
Method 3 − The ASF distribution with the largest median is used as the representative "mixture" ASF distribution to represent the chemical.¶

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Methods to compare early vs. later life cancer potencies from single-lifestage exposure studies, as illustrated by the DEN and ENU case studies, necessarily proceed differently from the methods described above for the multi-lifestage exposure studies. For DEN and ENU, there are several single-lifestage exposure experiments for each lifestage.

For each chemical, an overall distribution of the logarithm of potencies is created for each lifestage. This is accomplished via Monte Carlo methods, by sampling from each of the individual (log) potency distributions derived for each experiment for that exposure period equally to create an overall potency distribution for that lifestage. Overall potency distributions for the different lifestages are used to create a distribution of the ratio of the prenatal to juvenile potencies, and similarly for the postnatal to juvenile potencies, i.e., prenatal LP_i ratio distributions and postnatal LP_i ratio distributions.

Sensitivity analyses were also conducted, employing three alternative sampling methods to create the potency distribution for a given lifestage. One alternative method truncated each individual potency distribution at the fifth and ninety-fifth percentiles prior to creating the equally weighted potency mixture distribution. This eliminates the most extreme values from each potency distribution. A second alternative method did the same except it used the 25th and 75th percentiles. A third method sampled from the potency distributions based upon weights equal to the computed inverse-variance of each (logarithm) potency distribution. That is, the variance is calculated for the distribution of the logarithm of the q_1, Var[log q_1]. The likelihood that an q_1 is sampled is proportional to 1/Var(log[q_1]).

By using one of these methods, a potency mixture distribution for each lifestage is obtained. The ratio of mixture potency distributions for a given early lifestage (e.g., prenatal or postnatal) to the potency distribution for the juvenile lifestage is computed to arrive at the LP ratio distribution for that early lifestage. In general, exposures during the juvenile lifestage are expected to result in greater sensitivity to carcinogens than adult exposures, thus the LP ratios calculated here should be considered underestimates of the true LP ratio (i.e, the ratio of early to adult potencies) for these chemicals.

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Results

Here we present the results of analyses of data from the multi-lifestage exposure studies listed in Table 4 and from the single-lifestage exposure studies in mice used in the chemical-specific case studies of DEN and ENU listed in Table 5. In the case of the multi-lifestage exposure studies analyses, LP ratio distributions derived from individual studies within each early lifestage are presented, as well as prenatal, postnatal, and juvenile LP ratio mixture distributions and ASF mixture distributions representative of those for the chemicals studied in each of these early lifestages. For the DEN and ENU case studies, cancer potency distributions for each of the individual single-lifestage exposure experiments are presented, and then LP_a ratio mixture distributions, representing the ratio of prenatal to juvenile potency, and the ratio of postnatal to juvenile potency. These ratios are derived as distributions, representing the uncertainty in potency and variability in sensitivity of the animal strains on which these potencies are based. ASF_j mixture distributions, which represent both the inherent sensitivity of developing animals and the available time since exposure to develop cancer, are also presented for the DEN and ENU case studies.

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Prenatal Multi-Lifestage Exposure Studies

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Prenatal Study Specific LP Ratios

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Prenatal LP ratio distributions were generated for each of 22 multi-lifestage exposure studies extracted from the 16 publications with prenatal exposure groups listed in Table 4. Fourteen unique carcinogens are covered. Six of the 14 chemicals have two datasets representing each chemical and one chemical, ENU, has three. Figure 6 displays the prenatal LP ratio distributions for these studies. They are plotted on a logarithmic scale as "box plots," with upper 75th and lower 25th percentiles as the upper and lower edges of the boxes and triangles, and the upper 95% and lower 5% bounds as horizontal marks above and below the edges of the box. Appendix B, Table B1, gives the numerical values for these bounds, along with the mean and median for each of the displayed distributions.

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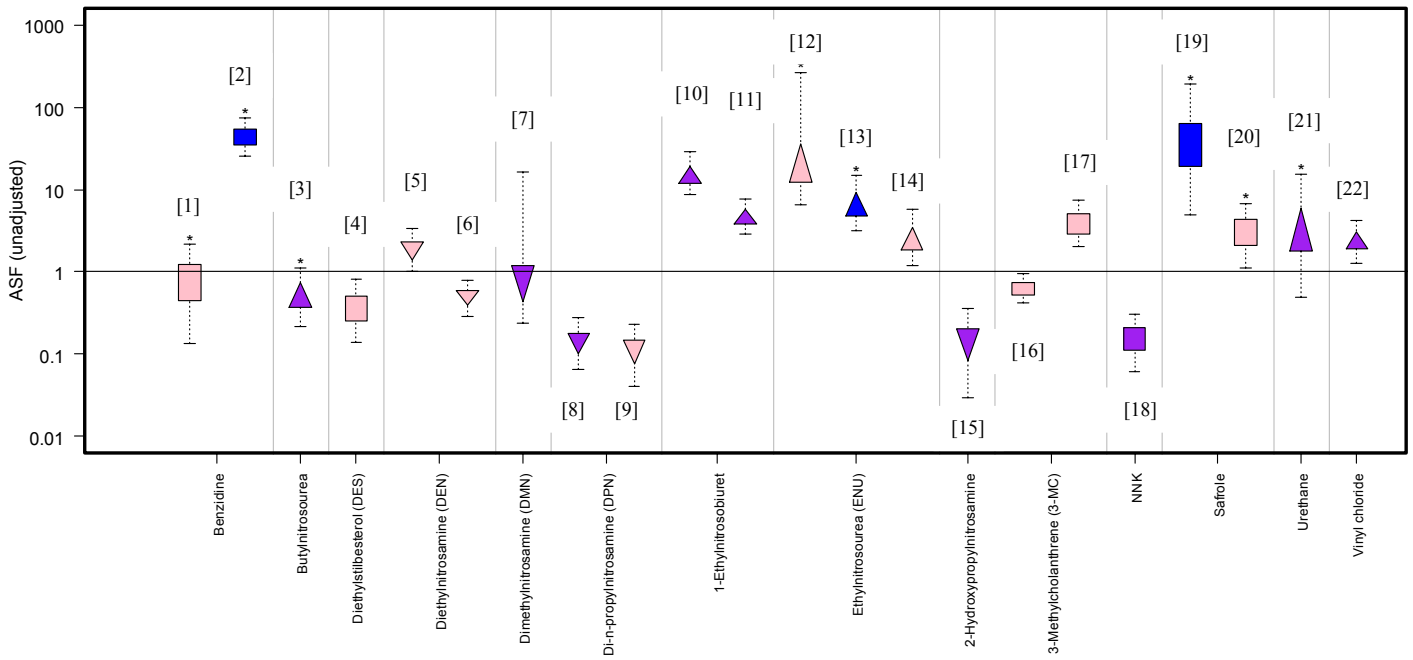
Figure 6. Prenatal LP Ratio Distributions



1. Vesselinovitch et al. (1979a), mouse, B6C3F1, F, day -9 to 21
2. Ibid, M, day -9 to 21
3. Zeller *et al.* (1978), rat, Sprague Dawley, M/F day -2
4. Turusov *et al.* (1992), mouse, CBA, F, day -2
5. Mohr et al. (1975), hamster, Syrian Golden, day -15 to -1
6. Mohr *et al.* (1995), hamster, Syrian Golden, F, day -3
7. Althoff *et al.* (1977), hamster, Syrian Golden, M/F, day -9 to -3
8. Ibid, day -9 to -3
9. Althoff and Grandjean (1979), hamster, Syrian Golden, F, day -9 to -3
10. Druckrey and Landschutz (1971), rat, BD IX, M/F, day -10
11. Ibid, day -3
12. Naito *et al.* (1981), rat, Wistar, day -9
13. Ibid, day -9
14. Tomatis *et al.* (1977), rat, BDVi, F, day -5
15. Althoff and Grandjean (1979), hamster, Syrian Golden, M/F, day -9 to -3
16. Tomatis *et al.* (1971), mouse, CF-1, F day -4 to -1
17. Turusov *et al.* (1973), mouse, CF-1, F, day -2
18. Anderson *et al.* (1989), mouse, C3H & B6C3 F_1,M/F day -8 to -4
19. Vesselnovitch *et al.* (1979a), mouse, B6C3 F_1, M, day -9 to -3
20. Vesselnovitch *et al.* (1979b), mouse, B6C3 F_1, F day -9 to -3
21. Choudari Kommineni *et al.* (1970), rat, MRC, M/F, day -4
22. Maltoni *et al.* (1981), rat, Sprague Dawley, M/F day -13 to -7

*LP ratio calculation is based on juvenile potency distribution

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Considerable variability in prenatal sensitivity is evident for the 14 carcinogens, with several demonstrating an enhanced tumor response, a few indicating an equivalent response, and others demonstrating a reduced tumor response associated with prenatal exposure as compared to adult exposure. The prenatal LP ratio 90% confidence intervals included values less than 0.1 for di-n-propylnitrosamine (based on studies in hamsters), 2-hydroxypropylnitrosamine (hamsters), and NNK (mice), values greater than 10 but less than 100 for benzidine (male mice), 1-ethylnitrosobiuret (rats), ENU (male rats), and urethane (rats), and values greater than 100 for ENU (female rats) and safrole (male mice). Twelve of the prenatal LP ratio distributions, representing studies of eight carcinogens, had medians that exceed unity. The remaining ten distributions, representing studies of nine carcinogens, had medians that were less than one.

Prenatal LP Ratio Mixture Distributions (LP Ratios for 14 Chemicals Combined)

The LP ratio mixture distributions characterize and summarize the prenatal LP ratio distributions from the prenatal multi-lifestage exposure studies on 14 chemicals displayed in Figure 6. As described in greater length in the Methods section above, in these derivations a single LP ratio distribution was obtained for each chemical, and then each was equally sampled to obtain the LP ratio mixture distribution.

Figure 7 displays the prenatal LP ratio mixture cumulative distribution functions, where Method 1 represents equal weighting of studies within a chemical, and Method 2 (inverse-variance weighting) and Method 3 (LP ratio distribution with the largest median selected for each chemical) represent the alternative weighting methods employed in the sensitivity analysis. In each case, these prenatal LP ratio distribution functions are essentially bimodal, with significant portions of each of the distributions below and above 1.0.

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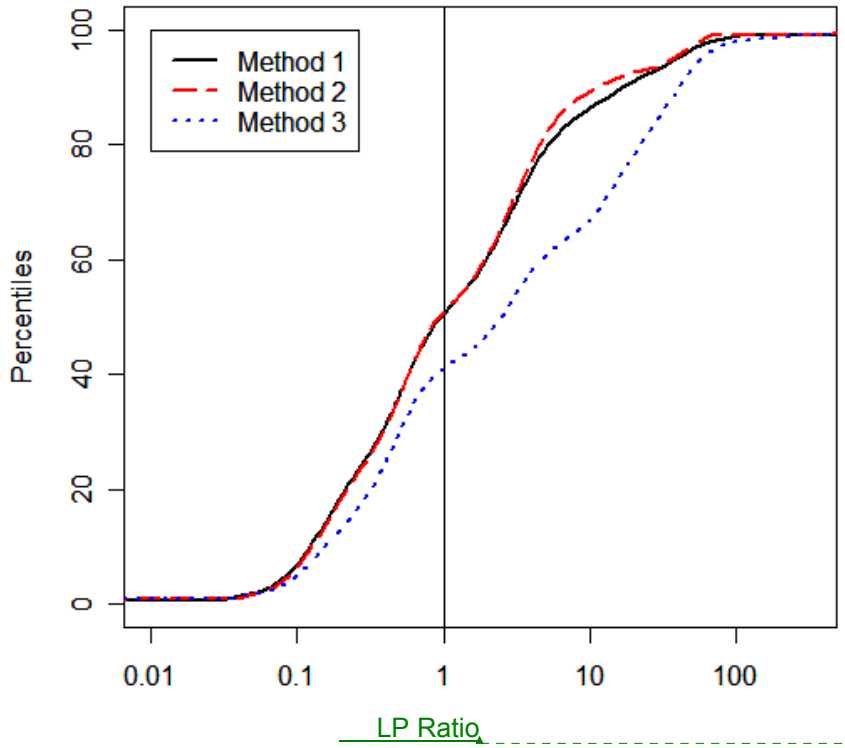
Figure 7. Prenatal LP Ratio Mixture Cumulative Distribution Functions



The mean and specific percentiles of the prenatal LP ratio mixture distribution and the prenatal ASF mixture distribution are provided in Table 6. The distributions are discussed in more detail in Appendix C, which also presents the results of the sensitivity analyses employing alternative sampling methods in cases where there were multiple studies on a chemical.

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Table 6. Prenatal LP Ratio and ASF Mixture Distribution Statistics

Statistics	LP Ratio	ASF
Mean*	7.03	21.09
Percentiles		
5th	0.09	0.27
10th	0.12	0.36
20th	0.22	0.66
30th	0.38	1.14
40th	0.58	1.74
50th	0.96	2.88
60th	1.95	5.85
70th	3.11	9.33
80th	5.18	15.54
90th	16.52	49.56
95th	38.49	115.47

* Calculated excluding large values above the 99th percentile.

Figure 8 shows the individual prenatal ASF 90% confidence bounds for each of the datasets used in generating the prenatal ASF mixture frequency distribution. The ASF 90% confidence bounds are displayed as a cumulative frequency profile.

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Figure 8. Prenatal ASF Cumulative Frequency Profile*

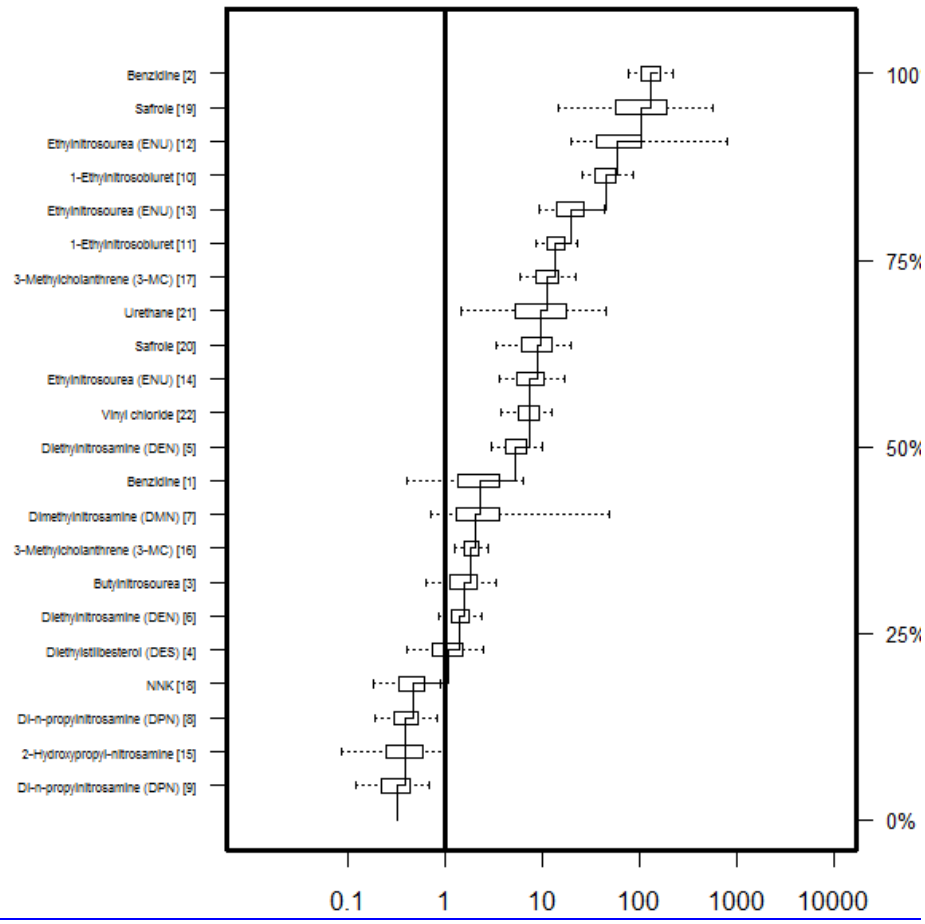

*Figure has same numbering of studies as in Figure 6 legend.

To summarize, the inherent sensitivity of animals to prenatal exposures to the carcinogens examined here appears dependent on the carcinogen and the animal species, sex, and strain, as is indicated by the prenatal LP ratio mixture distribution shown in Figure 7. For some chemicals, the animals were less susceptible in utero compared to adult exposure, and for a number of other cases just the opposite was observed. The prenatal ASF mixture distribution (shown in Figure 9), takes into account both the inherent sensitivity of prenatal animals and the available time since exposure to develop cancer. The majority of the distribution lies above an ASF of 1.0, indicating substantial susceptibility early in life (Figure 9).

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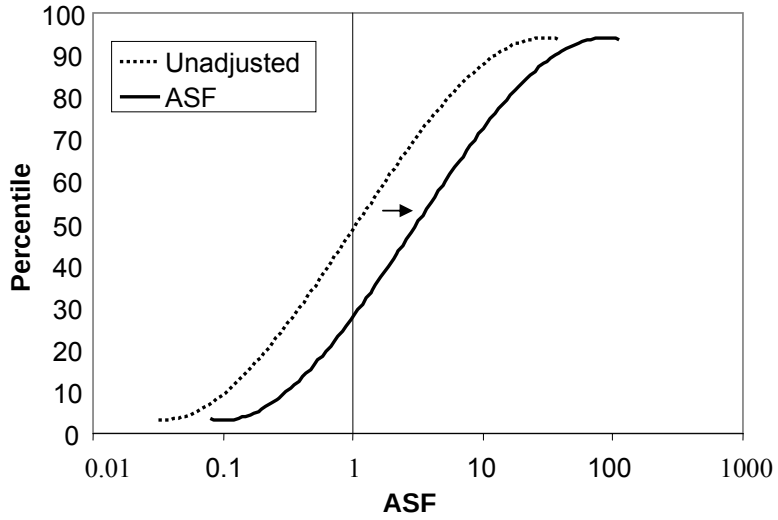
Figure 9. Prenatal ASF Mixture Distribution



Postnatal Multi-Lifestage Exposure Studies

Postnatal Study Specific LP Ratios

Postnatal LP ratio distributions generated for each of 55 multi-lifestage exposure studies are displayed in Figure 10. These studies were extracted from the 25 publications listed in Table 4 that included a postnatal exposure group. Eighteen unique carcinogens are represented. Eleven of the 18 chemicals have two or more datasets representing them. Figure 10 displays the LP ratios for these studies as box plots. The values associated with the mean, 5th, 25th, 50th, 75th, and 95th percentile values for each of the LP ratios shown in the figure are given in Appendix B, Table B2.

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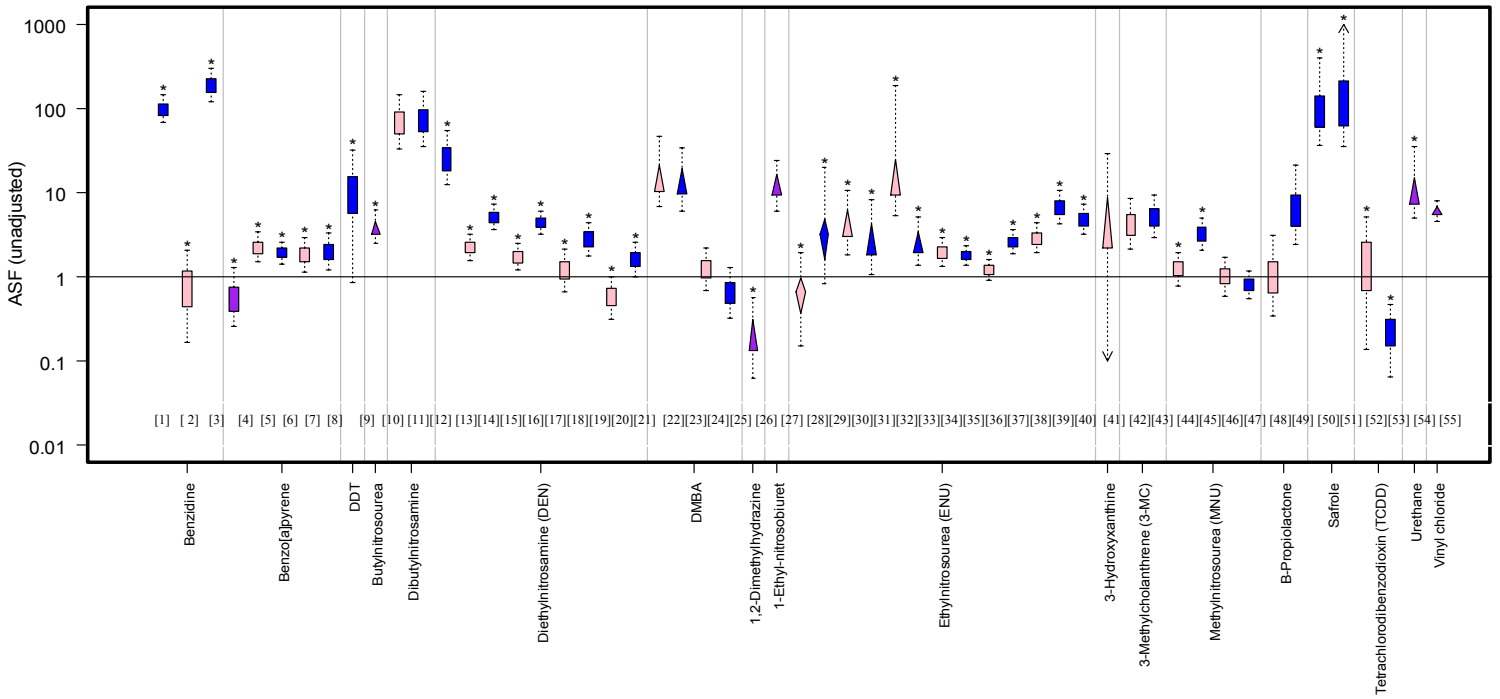
Figure 10. Postnatal LP Ratio Distributions [1]



[1] Bracketed numbers indicate study reference and characteristics given on next page
Upper 75th and lower 25th percentiles are the upper and lower edges of the boxes and triangles, and the upper 95% and lower 5% bounds as horizontal marks above and below the edges of the box.
*LP ratio calculation is based on juvenile potency distribution

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Figure 10 (continued): Studies Identifiers

1 Vesselinovitch *et al.* (1975b), mouse, B6C3F$_1$, M, day 7-27
2 Vesselinovitch *et al.* (1979), mouse, B6C3F$_1$, F, day 1-21
3 Ibid, M, day 1-21
4 Truhaut *et al.* (1966), mouse, swiss, M/F, day 1
5 Vesselinovitch *et al.* (1975a), mouse, B6C3F$_1$, F, day 1
6 Ibid, M, day 1
7 Ibid, C3A F$_1$, F, day 1
8 Ibid, M, day 1
9 Vesselinovitch *et al.* (1979a), mouse, B6C3F$_1$, M, day 1-28
10 Zeller *et al.* (1978), rat, Sprague Dawley, M/F, day 2
11 Wood *et al.* (1970), mouse, IF x C57, F, day 1-15
12 Ibid, M, day 1-15
13 Rao and Vesselinovitch (1973), mouse, B6C3F$_1$, M, day 15
14 Vesselinovitch *et al.* (1984), mouse, B6C3F$_1$, F, day 1
15 Ibid, M, day 1
16 Ibid, F, day 15
17 Ibid, F, day 15
18 Ibid, C3A F$_1$, F, day 1
19 Ibid, M, day 1
20 Ibid, F, day 15
21 Ibid, M, day 15
22 Meranze *et al.* (1969), rat, Fels-Wistar, F, day 10
23 Ibid, M, day 10
24 Walters (1966), mouse, BALB/c, F, day 17
25 Ibid, M, day 17
26 Martin *et al.* (1974), rat, BDIX, M/F, day 10
27 Druckrey and Landschutz (1971), rat, BDIX, M/F, day 10
28 Naito *et al.* (1985), gerbil, mongolian, F, day 1
29 Ibid, M, day 1
30 Bosch (1977), rat, WAG, F, day 8
31 Ibid, M, day 8
32 Naito *et al.* (1981), rat, Wistar, F, day 7
33 Ibid, M, day 7
34 Vesselinovitch *et al.* (1974), mouse, B6C3F$_1$, F, day 1
35 Ibid, M, day 1
36 Ibid, F, day 15
37 Ibid, M, day 15
38 Ibid, C3A F$_1$, F, day 1
39 Ibid, M, day 1
40 Ibid, M, day 15
41 Anderson *et al.* (1978), rat, Wistar, F, day 9
42 Klein (1959), mouse, A/He, F, day 8-31
43 Ibid, M, day 8-31
44 Terracini and Testa (1970), mouse, B6C3F$_1$, F, day 1
45 Ibid, M, day 1
46 Terracini *et al.* (1976), mouse, C3Hf/Dp, F, day 1
47 Ibid, M, day 1
48 Chernozemski and Warwick (1970), mouse, B6A F$_1$, F, day 9
49 Ibid, M, day 9
50 Vesselinovitch *et al.* (1979a), mouse, B6C3F$_1$, M, day 1-21
51 Vesselinovitch *et al.* (1979b), mouse, B6C3F$_1$, M, day 1-21
52 Della Porta *et al.* (1987), mouse, B6C3F$_1$, F, day 10-45
53 Ibid, M, day 10-45
54 Choudari Kommineni *et al.* (1970), rat, MRC, M/F, day 1-17
55 Maltoni *et al.* (1981), rat, Sprague Dawley, M/F, day 1-35

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For two-thirds of the studies plotted - thirty-seven postnatal datasets (for 15 carcinogens) – the LP ratio distributions are significantly greater than unity (i.e., the lower 95% confidence bound exceeds unity). For sixteen postnatal studies or 29% of the total, representing nine carcinogens, 90% confidence intervals straddle unity. Two postnatal studies, or only 4% of the plotted studies, representing two carcinogens, have LP ratios with upper 95% confidence bounds less than unity.

Postnatal LP Ratio Mixture Distributions (LP Ratios for 18 Chemicals Combined)

The LP ratio mixture distributions characterize and summarize the postnatal LP ratio distributions from the postnatal multi-lifestage exposure studies on 18 chemicals displayed in Figure 10. In these derivations, a single LP ratio distribution was obtained for each chemical and then each chemical was equally sampled to obtain the LP ratio mixture distribution (see Methods).

Figure 11 displays the postnatal LP ratio mixture cumulative distribution functions, where Method 1 represents equal weighting of studies within a chemical, and Method 2 (inverse-variance weighting) and Method 3 (LP ratio distribution with the largest median selected for each chemical) represent the alternative weighting methods employed in the sensitivity analysis.

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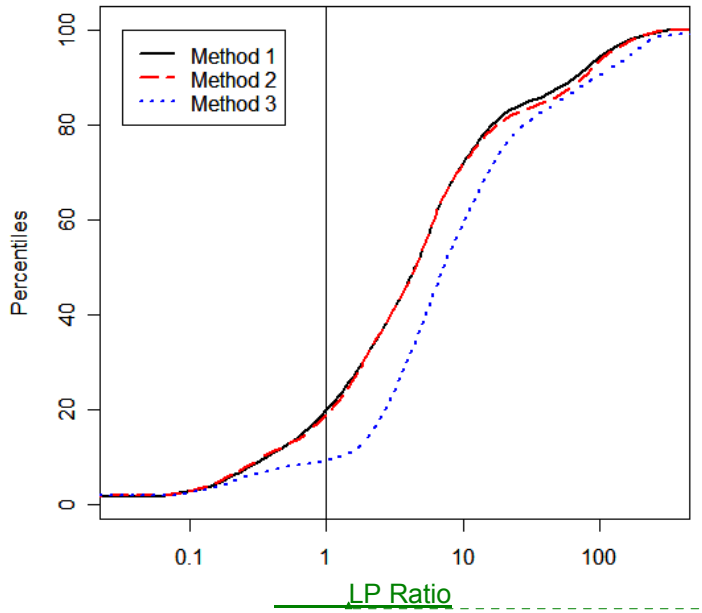
Figure 11. Postnatal LP Ratio Mixture Cumulative Distribution Functions



The mean and specific percentiles of the postnatal LP ratio and ASF mixture distributions are provided in Table 7. The distributions are discussed in more detail in Appendix C, which also presents the results of the sensitivity analyses employing alternative sampling methods in cases where there were multiple studies on a chemical.

Table 7. Postnatal LP Ratio and ASF Mixture Distribution Statistics

Statistics	LP Ratio	ASF
Mean*	27.08	78.53
Percentiles		
5th	0.20	0.58
10th	0.41	1.19
20th	1.08	3.13
30th	1.93	5.60
40th	3.13	9.08
50th	4.64	13.46
60th	6.35	18.42
70th	9.62	27.90
80th	18.10	52.49
90th	72.78	211.06
95th	122.82	356.18

* Calculated excluding large values above the 99th percentile.

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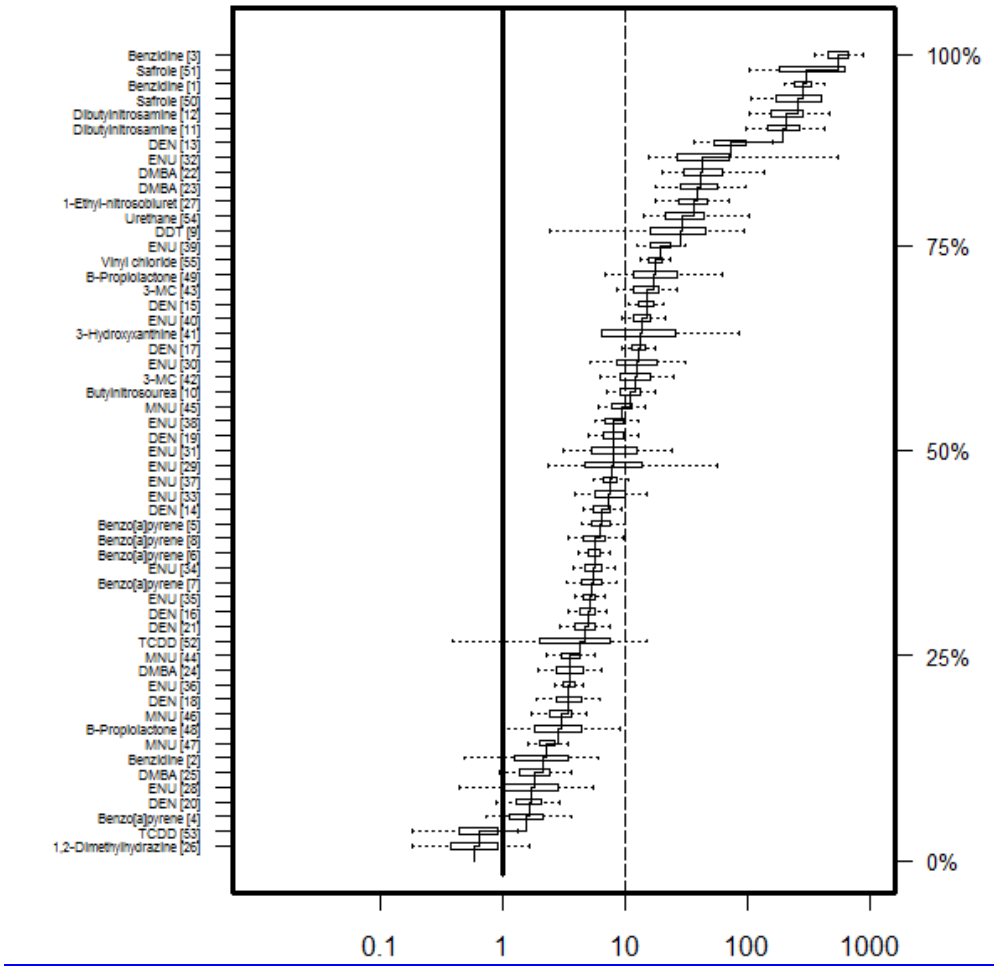
Figure 12 shows the individual postnatal ASF 90% confidence bounds for each of the datasets used in generating the postnatal ASF mixture frequency distribution. The ASF 90% confidence bounds are displayed as a cumulative frequency profile.

Figure 12. Postnatal ASF Cumulative Frequency Profile*



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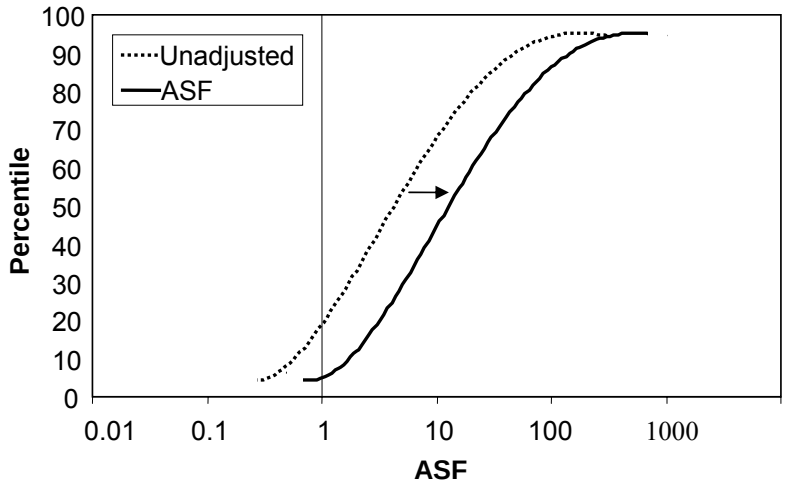
To summarize, in general for the cases studied here animals are inherently more sensitive in the postnatal period, as indicated by the postnatal LP ratio mixture distribution (Figure 11). The postnatal ASF mixture distribution shown in Figure 13 below takes into account both the inherent sensitivity of postnatal animals and the available time since exposure to develop cancer. The majority of the distribution lies above an ASF of 1.0, indicating substantial susceptibility early in life (Figure 13).

Figure 13. Postnatal ASF Mixture Distribution



Juvenile Multi-Lifestage Exposure Studies

Juvenile Study Specific LP Ratios

Juvenile LP ratio distributions were generated for each of seven multi-lifestage exposure studies extracted from five publications with juvenile and adult exposure groups, covering five unique carcinogens (See Table 4). Figure 14 displays the juvenile LP ratio distributions in boxplot form. Appendix B, Table B3, provides the mean, 5th, 25th, 50th, 75th, and 95th percentile values for these LP ratios. All studies were conducted in rats. Four studies have juvenile LP ratios significantly greater than unity ($p \leq 0.05$), and the 90% confidence interval straddles unity for the remaining three studies. Of the two LP ratio distributions representing the chemical MNU from

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the publication of Grubbs *et al.* (1983), only one is used in determining the juvenile LP ratio mixture distribution, since the two LP ratio distributions are not independent. The juvenile exposure data (representing the numerator of both LP ratio distributions) are from the same group of female rats exposed on days 50 through 57, but the adult exposure data (representing the denominators of the LP ratio distributions) differ. In the first MNU juvenile LP ratio distribution the adult exposure data are from females exposed on days 80 through 87. In the second MNU juvenile LP ratio distribution the adult exposure data are from females exposed on days 140 through 147. These MNU data illustrate that even within the adult lifestage, the earlier the exposure occurs, the more sensitive the animal is to MNU-induced mammary tumors. For DMBA, the juvenile females are significantly more sensitive than the adult animals, as reflected in the LP ratio distribution significantly exceeding unity. For juvenile DMBA exposed males there is no significant difference with adults and the LP ratio is consistent with unity.

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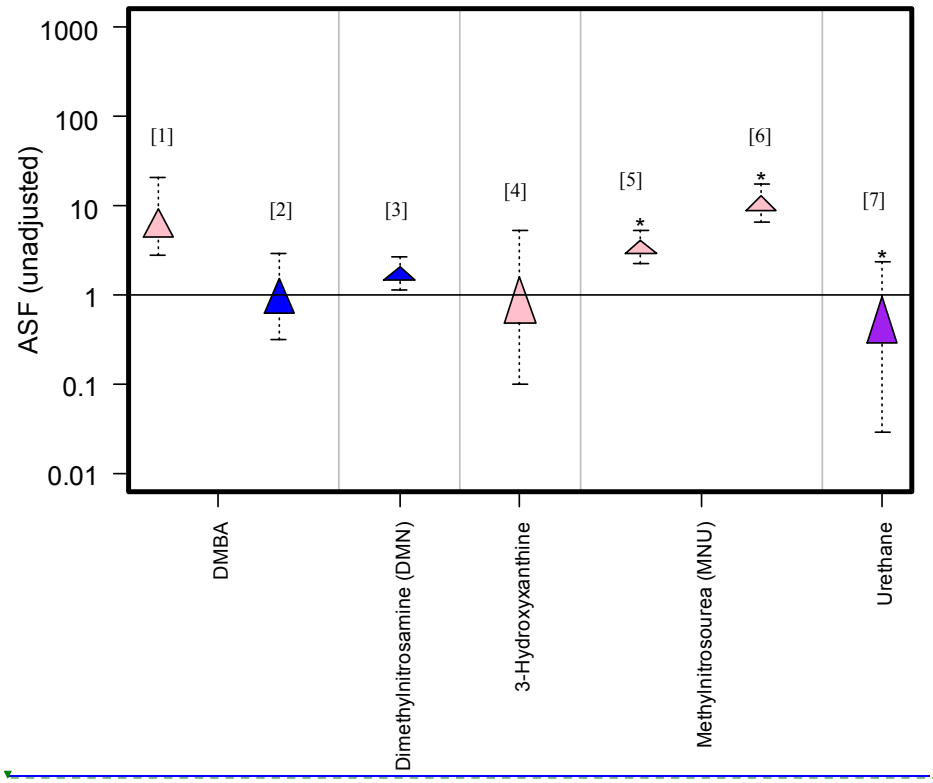
Figure 14. Juvenile LP Ratio Distributions



♦ Adult comparison group dosed on days 80-87
+ Adult comparison group dosed on days 140-147
* Adult comparison group dosing began during late juvenile lifestage (day 46) and continued through day 61

1. Meranze *et al.* (1969), rat, Fels-Wistar, F, day 45
2. Ibid, M, day 451
3. Noronha and Goodall (1984), rat, CRL/CDF, M, day 46
4. Anderson *et al.* (1978), rat, Wistar, F, day 28
5. Grubbs *et al.* (1983), rat, Sprague Dawley, F, day 50-57
6. Ibid, M, day 50-57
7. Choudari Kommineni et al. (1970), rat, MRC, M/F, day 28-43

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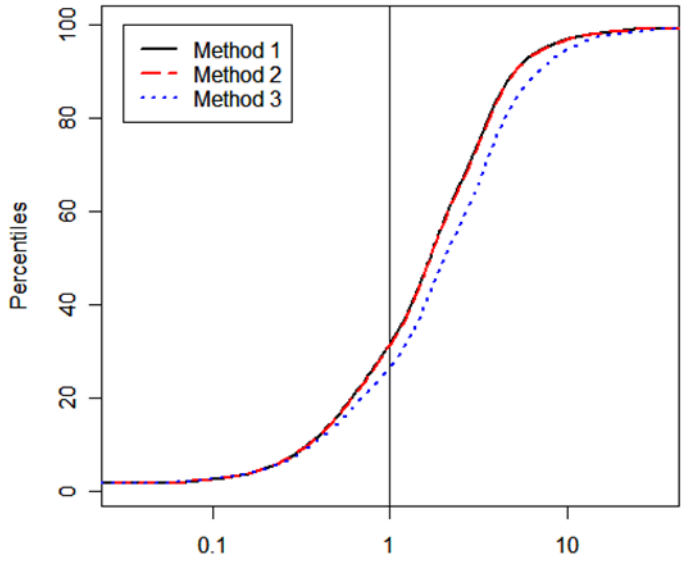
Juvenile LP Ratio Mixture Distributions (LP Ratios for Five Chemicals Combined)

The LP ratio mixture distributions characterize and summarize the juvenile LP ratio distributions from the juvenile multi-lifestage exposure studies on five chemicals displayed in Figure 14. In these derivations, a single LP ratio distribution was obtained for each chemical and then each chemical was equally sampled to obtain the LP ratio mixture distribution (see Methods).

Figure 15 displays the juvenile LP ratio mixture cumulative distribution functions, where Method 1 represents equal weighting of studies within a chemical, and Method 2 (inverse-variance weighting) and Method 3 (LP ratio distribution with the largest median selected for each chemical) represent the alternative weighting methods employed in the sensitivity analysis. Since only one chemical, DMBA, had more than one study and the LP ratio differences for this chemical were moderate, the three methods produced similar results.

Figure 15. Juvenile LP Ratio Mixture Cumulative Distribution Functions



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4. Anderson *et al*. (1978), rat, Wistar, F, day 28¶
5. Grubbs *et al*. (1983), rat, Sprague Dawley, F, day 50-57¶
6. Ibid, M, day 50-57¶
7. Choudari Kommineni *et al*. (1970), rat, MRC, M/F, day 28-43¶

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The mean, and certain percentiles of the juvenile LP ratio mixture distribution and the juvenile ASF mixture distributiona are provided in Table 8. The distributions are discussed in more detail in Appendix C, which also presents the results of the sensitivity analyses employing alternative sampling methods in cases where there were multiple studies on a chemical.

Table 8. Juvenile LP Ratio and ASF Mixture Distribution Statistics

Statistics	LP Ratio	ASF
Mean*	2.63	7.10
Percentiles		
5th	0.20	0.54
10th	0.34	0.92
20th	0.60	1.62
30th	0.93	2.51
40th	1.31	3.54
50th	1.67	4.51
60th	2.10	5.67
70th	2.77	7.48
80th	3.57	9.64
90th	4.96	13.39
95th	7.29	19.68

* Calculated excluding large values above the 99th percentile.

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Figure 16 shows the individual juvenile ASF 90% confidence bounds for each of the datasets.

The ASF 90% confidence bounds are displayed as a cumulative frequency profile.

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Figure 16. Juvenile ASF Cumulative Frequency Profile

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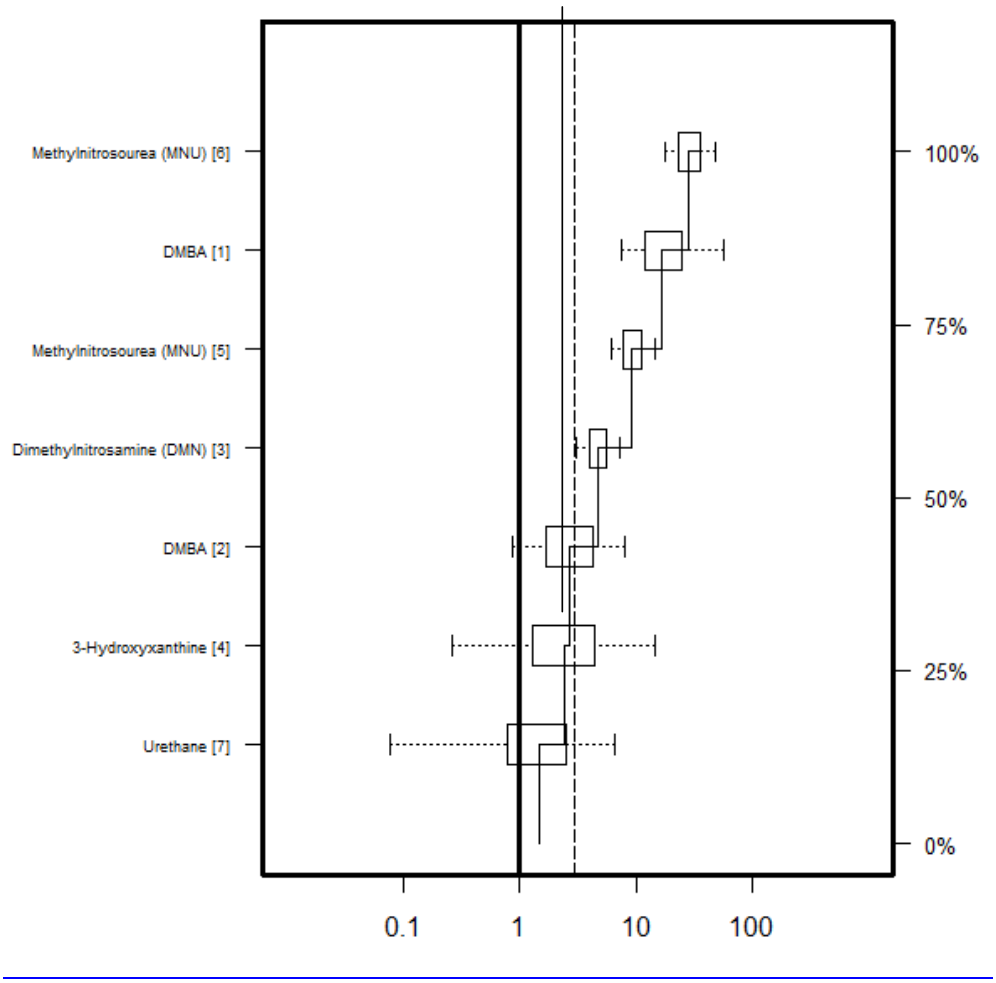

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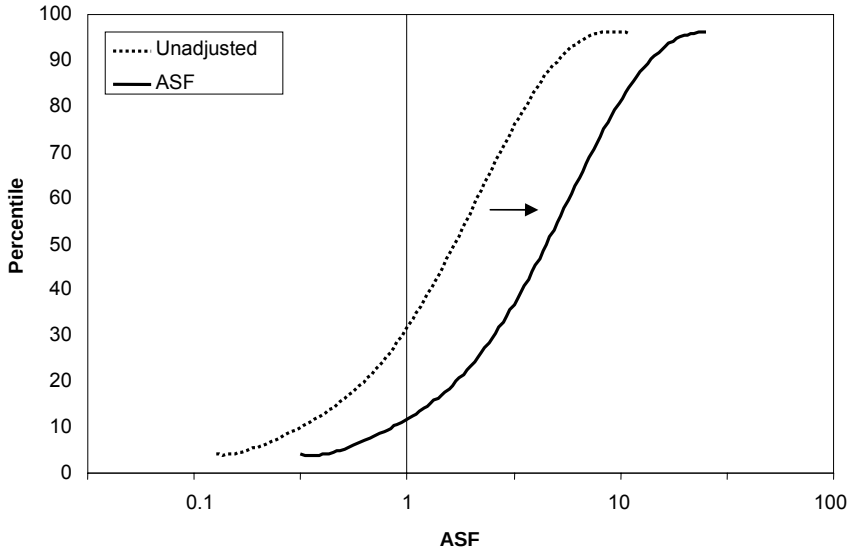
Based on the limited data set analyzed here, animals are inherently more sensitive in the juvenile period, as indicated by the juvenile LP ratio mixture distribution (Figure 15). The juvenile ASF mixture distribution shown in Figure 17 below takes into account both the inherent sensitivity of juvenile animals and the available time since exposure to develop cancer. The majority of the distribution lies above an ASF of 1.0, indicating susceptibility early in life.

Figure 17. Juvenile ASF Mixture Distribution



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DEN Case Study

Ten mouse publications on DEN were included in the compilation of single-lifestage exposure studies in mice (See Table 5). Of these, three included groups of mice exposed during the prenatal lifestage, seven included groups of mice exposed during the postnatal lifestage, and two included groups of mice exposed during the juvenile lifestage. These studies yielded a total of eight prenatal datasets, 18 postnatal datasets, and five juvenile datasets. No "adult only" exposure studies were identified in mice for DEN. Thus the juvenile exposure studies were used as the "later life" exposure comparison group. As noted earlier, if mice exposed to DEN during the juvenile lifestage are more prone to cancer than fully mature animals exposed to DEN, then the use of these juvenile exposure studies as the comparison group will result in an overall underestimate of the comparative cancer susceptibility of exposures during the prenatal and postnatal periods.

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Cancer Potency Distributions

Figure 18 displays the box plots representing the cancer potencies derived for the different DEN prenatal, postnatal and juvenile single-lifestage exposure studies in the mouse. The interquartile range of the potency distributions is shown as boxes, while the upper and lower bars extend from the box to the 95th and 5th percentiles, respectively. The Appendix D tables give the numerical values for these bounds, along with the mean, standard deviation, and median for each of the displayed distributions. The prenatal potency distributions fall into two distinct groupings. One grouping is located about the potency value 0.1. The second grouping is centered approximately at the potency value 0.005. The second grouping of studies exhibits greater fold-variability than the first grouping. The postnatal potency distributions all have confidence intervals that are entirely above the potency value of 0.1. Graphically, a greater cancer risk for mice exposed during the postnatal lifestage as compared to the prenatal lifestage is apparent. The juvenile potency distributions also have slightly elevated potency values compared to those derived from the prenatal studies.

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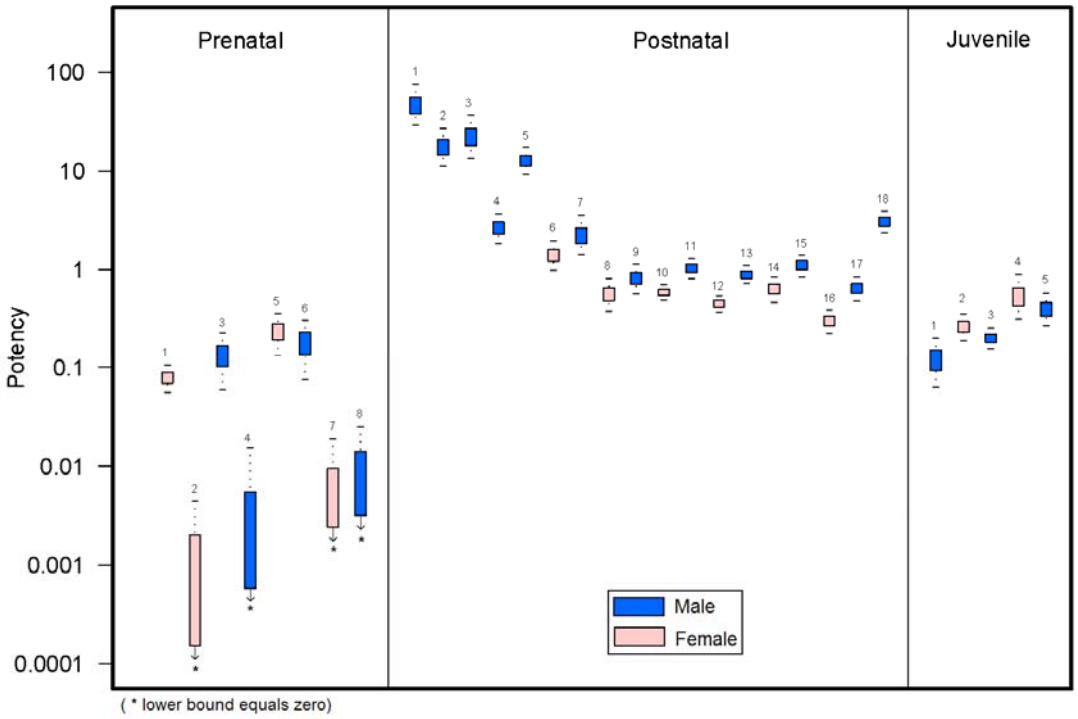
Figure 18. Cancer Potencies for DEN in Mice Exposed during the Prenatal, Postnatal or Juvenile Lifestages



Bracketed numbers indicate study reference and characteristics given on next page
Upper 75th and lower 25th percentiles are the upper and lower edges of the boxes and triangles, and the upper 95% and lower 5% bounds as horizontal marks above and below the edges of the box

Prenatal Exposure

1 Anderson *et al.* (1989), C3H/HeN, F, sac day 540
2 Ibid, sac day 650
3 Ibid, M, sac day 461
4 Ibid, sac day 644
5 Mohr and Althoff (1965), NMRI, F
6 Ibid, M
7 Vesselinovitch (1983), $B6C3F_1$, F
8 Ibid, M

Postnatal Exposure

1 Boberg *et al.* (1983), $B6C3F_1$, M
2 Drinkwater and Ginsler (1986), $B6C3F_1$, M
3 Ibid, C3H/HeJ, M
4 Ibid, C57BL/6J, M
5 Lai *et al.* (1985), $B6C3F_2$, M
6 Rao and Vesselinovitch (1973), $B6C3F_1$, F
7 Ibid, M
8 Turusov *et al.* (1973), CF-1, F
9 Ibid, M
10 Vesselinovitch *et al.* (1984), $B6C3F_1$, F, day 1
11 Ibid, M, day 1
12 Ibid, F, day 15
13 Ibid, M day 15
14 Ibid, $C3AF_1$, F, day 1
15 Ibid, M, day 1
16 Ibid, F, day 15
17 Ibid, M, day 15
18 Vesselinovitch. (1980), $B6C3F_1$, M

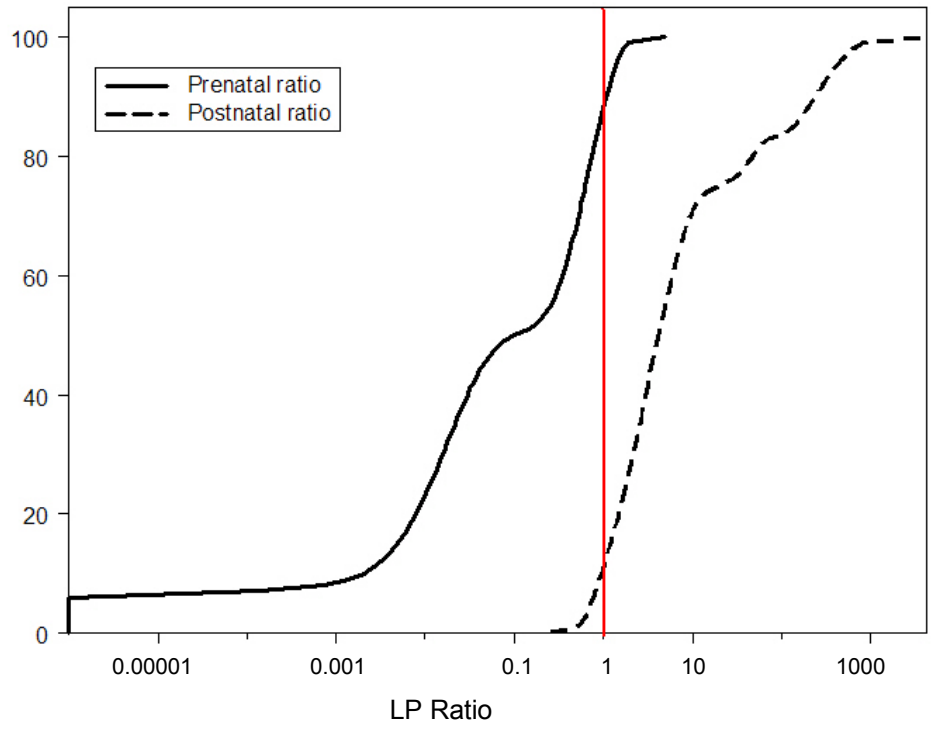
Juvenile Exposure

1 Rao and Vesselinovich (1973), $B6C3F_1$, M
2 Vesselinovitch *et al.* (1984), $B6C3F_1$, F
3 Ibid, M
4 Ibid, $C3AF_1$, F
5 Ibid, M

DEN Case Study: Prenatal and Postnatal LP_i Ratio Distributions

Mixture potency distributions were calculated for the eight prenatal DEN exposure studies, the 18 postnatal DEN exposure studies, and the five juvenile DEN exposure studies. The differences in sensitivity to DEN among the prenatal and postnatal lifestages is evident, with animals exposed in utero exhibiting considerably less sensitivity than those exposed postnatally.

Figure 19. DEN Prenatal and Postnatal LP_i Ratio Cumulative Distribution Functions – Equal Weighting of Potency Distributions



The percentiles for the prenatal and postnatal LP_i ratio distributions are provided in Table 9. The 88th percentile of the prenatal LP_i ratio distribution is slightly less than unity. The distributional statistics indicate that mice exposed during the prenatal lifestage are less prone to the tumorigenic effects of DEN as compared to those exposed as juveniles. In contrast, the 11th percentile of the postnatal LP_i ratio distribution is greater than unity, thus 89% of the distribution

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indicates that mice exposed during the postnatal lifestage are more prone to the tumorigenic effects of DEN than those exposed as juveniles. The distributional differences in cancer risk (as compared to juveniles) between DEN exposures occurring during the prenatal lifestage versus the postnatal lifestage are quite evident.

Table 9. DEN Prenatal and Postnatal LP_i Ratio Distributions

Percentiles	Prenatal LP_i Ratio	Postnatal LP_i Ratio
5th	0.00	0.74
10th	0.002	0.96
20th	0.008	1.50
30th	0.02	2.19
40th	0.03	3.00
50th	0.10	4.21
60th	0.35	6.01
70th	0.53	9.53
80th	0.75	47.51
90th	1.08	240.62
95th	1.36	408.95

Table 10 shows the DEN prenatal and postnatal ASF_i mixture distribution statistics. The distributions are discussed more in detail in Appendix D, which also presents the results of the sensitivity analyses employing alternative sampling methods to create the mixture potency distributions for the different lifestages. In this case, at approximately the 60th percentile, the DEN prenatal ASF_i indicates equal contribution to lifetime risk from juvenile and *in utero* exposure. The postnatal ASF_i indicates considerably greater contributions to risk from postnatal DEN exposures, as compared to juvenile exposures.

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Figure 15 shows the DEN prenatal and postnatal ratio frequency distributions generated using Method 1. Both the prenatal and postnatal ratio frequency distributions are multi-modal.¶
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Figure 15. Method 1 DEN Prenatal and Postnatal Ratio Frequency Distributions –Equal Weighting of Potency Distributions¶
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Table 10. DEN Prenatal and Postnatal ASF_i Mixture Distribution Statistics –

Percentiles	Prenatal ASF_i	Postnatal ASF_i
5th	0.00	2.14
10th	0.01	2.78
20th	0.02	4.34
30th	0.05	6.37
40th	0.09	8.70
50th	0.31	12.20
60th	1.05	17.43
70th	1.58	27.65
80th	2.24	137.79
90th	3.25	697.81
95th	4.07	1185.95

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Method 2: Weighting Potency Distributions by Inverse-Variance and the Interquartile Range.¶
Figure 16 shows the DEN prenatal and postnatal ratio cumulative distribution functions generated using Method 2a, weighting by inverse-variance, and Method 2b, weighting by the interquartile range (IQR). Qualitatively the results are similar to Method 1, with considerable sensitivity exhibited in the postnatal window. The magnitude of the differences in the ratio distributions for DEN across the prenatal and postnatal exposure windows is evident. ¶
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Figure 16. Methods 2a and 2b DEN Prenatal and Postnatal Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions¶
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ENU Case Study

Thirteen mouse publications on ENU were included in the compilation of single-lifstage exposure studies in mice (See Table 5). Of these, five included groups exposed during the prenatal lifestage, eight included groups exposed during the postnatal lifestage, and three included groups exposed during the juvenile lifestage. These studies yielded a total of 30 prenatal, 27 postnatal, and eight juvenile datasets. As with DEN, no "adult only" exposure studies were available and the juvenile exposure studies were used as the "later life" exposure comparison group.

Cancer Potency Distributions

Figure 20 displays box plots representing the cancer potencies derived for the different ENU prenatal, postnatal and juvenile single-lifestage exposure studies in the mouse. The interquartile range of the potency distributions is shown as boxes, while the upper and lower bars extend from the box to the 95th and 5th percentiles, respectively. The Appendix E tables give the numerical values for these bounds, along with the mean, standard deviation, and median for each of the

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displayed distributions. The prenatal potency distributions fall into two distinct groupings. One grouping is located about the potency value 4.0, and a second grouping is centered approximately at the potency value 0.1. The grouping of prenatal studies with potency values centered around 4.0 have greater variability than the prenatal studies centered around the lower potency value of 0.1. The postnatal potency distributions also exhibit two distinct groupings, with one grouping located about the potency value 0.7, and a second centered approximately at the potency value 0.1. The grouping of postnatal studies centered around 0.7 have greater variability than the postnatal studies centered around the lower potency value of 0.1. Finally, two distinct groupings are also apparent for the juvenile studies. One grouping is located about the potency value 0.05. The second grouping is centered approximately at the potency value 0.007. The grouping of juvenile studies centered about the potency value of 0.007 has greater variability than the grouping of juvenile studies centered about the higher potency value of 0.05.

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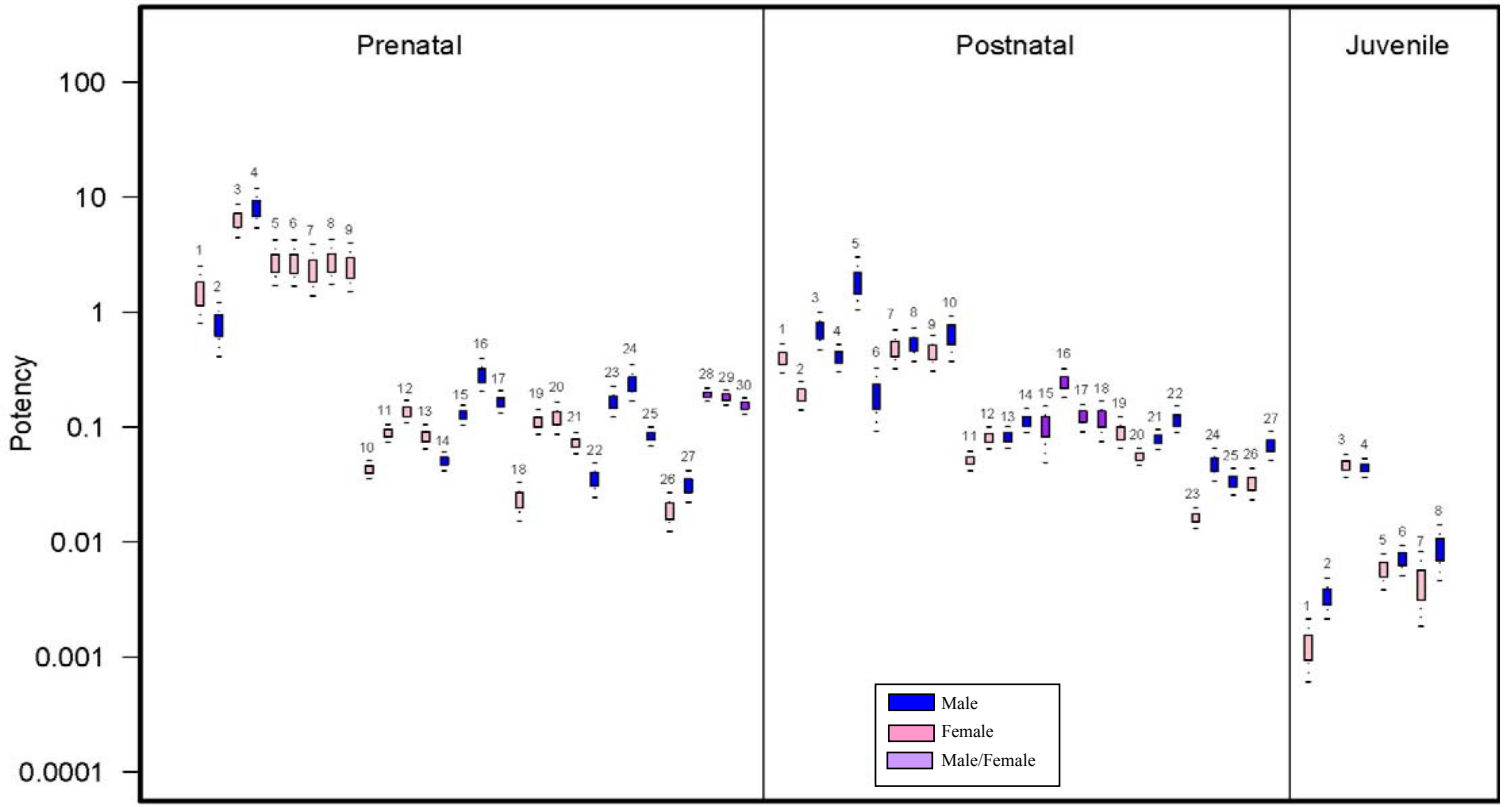
Figure 20. Cancer Potencies for ENU in Mice Exposed during the Prenatal, Postnatal or Juvenile Lifestages[1]



[1]Numbers indicate study reference and characteristics given on next page
Upper 75th and lower 25th percentiles are the upper and lower edges of the boxes and triangles, and the upper 95% and lower 5% bounds as horizontal marks above and below the edges of the box

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	Prenatal Exposure		*Postnatal Exposure*		*Juvenile Exposure*
1	Diwan *et al.* (1974), AKR/J x SW/J, M	1	Anderson *et al.* (1989), C3H/HenCr MTV,F, sac day 405	25	Ibid, day 15
2	Ibid, F	2	Ibid, sac day 451	26	Vesselinovitch (1983), B6C3F$_1$, F
3	Ibid, SW/J x AKR/J , M	3	Ibid, M, sac day 342	27	Ibid, M
4	Ibid, F	4	Ibid, sac day 397		
5	Kauffman (1976), Swiss, F, day -7	5	Drinkwater and Ginsler (1986), C3F/HeJ, M	1	Vesselinovitch *et al.* (1973), B6C3F$_1$, F
6	Ibid, day -6	6	Ibid, C57BL/6, M	2	Ibid, M
7	Ibid, day -5	7	Naito *et al.* (1982), A/He, F	3	Vesselinovitch *et al.* (1974), C3AF$_1$, F
8	Ibid, day -4	8	Ibid, M	4	Ibid, M
9	Ibid, day -3	9	Pereira *et al.* (1985), Cd1, F	5	Ibid, B6C3F$_1$, F
10	Vesselinovitch *et al.* (1977), B6C3F$_1$, day -10	10	Ibid, M	6	Ibid, M
11	Ibid, day -8	11	Schmahl (1988), NMRI, F	7	Vesselinovitch (1983), B6C3F$_1$, F
12	Ibid, day -6	12	Ibid, F (independent exp)	8	Ibid, M
13	Ibid, day -4	13	Ibid, M		
14	Ibid, day -10	14	Ibid, M (independent exp)		
15	Ibid, day -8	15	Searle and Jones (1976), A, M/F		
16	Ibid, day -6	16	Ibid, C57BL, M/F		
17	Ibid, day -4	17	Ibid, DBA, M/F		
18	Ibid, C3B6F$_1$, day -10	18	Ibid, IF, M/F		
19	Ibid, day -8	19	Vesselinovitch *et al.* (1974), B6C3F$_1$, F, day 1		
20	Ibid, day -6	20	Ibid, day 15		
21	Ibid, day -4	21	Ibid, M, day 1		
22	Ibid, day -10	22	Ibid, day 15		
23	Ibid, day -8	23	Ibid, C3AF$_1$, F		
24	Ibid, day- 6	24	Ibid, M, day 1		
25	Ibid, day -4				
26	Vesselinovitch (1983), B6C3F$_1$, F				
27	Ibid, M				
28	Wiggenhauser and Schmahl (1987), NMRI, day -8				
29	Ibid, day -7				
30	Ibid, day -6				

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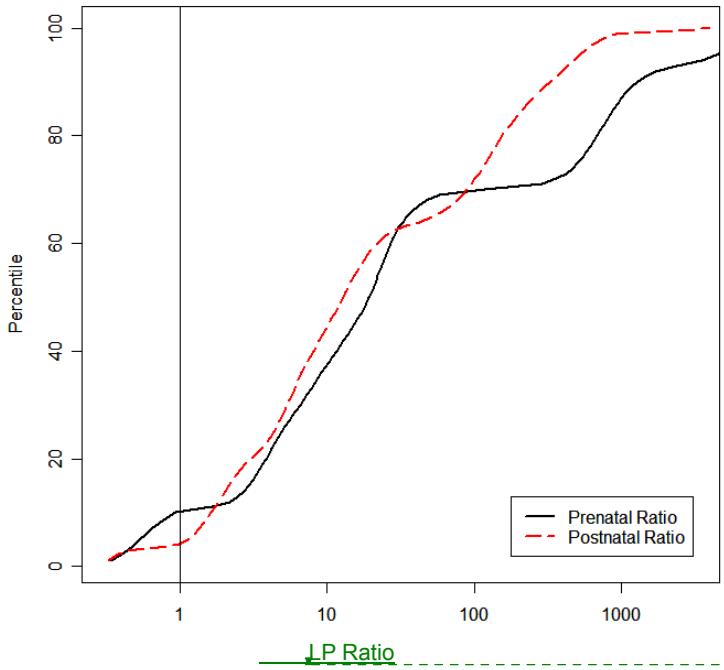
ENU Case Study: Prenatal and Postnatal LP_i Ratio Distributions

Using the same methods as described for DEN, mixture potency distributions were calculated for the 30 prenatal ENU exposure studies, the 27 postnatal exposure studies, and the eight juvenile exposure studies. These distributions were used to calculate prenatal and postnatal LP_i ratio distributions.

Figure 21 shows the ENU prenatal and postnatal LP_i ratio cumulative distribution functions generated by equally weighting each experiment within a given lifestage. In contrast to DEN, the sensitivity of mice to ENU in both the prenatal and postnatal lifestages is evident.

Figure 21. ENU Prenatal and Postnatal LP_i Ratio Cumulative Distribution Functions – Equal Weighting of Potency Distributions



The percentiles for the prenatal and postnatal LP_i ratio distributions are provided in Table 11. Almost ninety percent of the prenatal LP_i ratio distribution exceeds unity, twenty-eight percent is between unity and 10, and sixty-two percent is greater than 10. These observations indicate that mice exposed during the prenatal lifestage are more prone to the tumorigenic effects of ENU

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than those exposed as juveniles. In addition, more than 95% of the postnatal LP_i ratio distribution is greater than unity, indicating that mice exposed during this lifestage are more prone to the tumorigenic effects of ENU than those exposed as juveniles.

Table 11. ENU Prenatal and Postnatal LP_i Ratio Distributions

Percentiles	Prenatal LP_i Ratio	Postnatal LP_i Ratio
5th	0.53	1.14
10th	0.94	1.65
20th	3.86	3.03
30th	6.56	5.39
40th	11.60	8.09
50th	19.30	12.84
60th	27.13	21.87
70th	116.16	88.96
80th	679.56	154.90
90th	1266.12	325.80
95th	4381.63	519.75

Table 12 shows the ENU prenatal and postnatal ASFj mixture distribution statistics. The distributions are discussed in more detail in Appendix E, which also presents the results of the sensitivity analyses employing alternative sampling methods to create the mixture potency distributions for the different lifestages. The prenatal ASF_i and the postnatal ASF_i indicate considerably greater contributions to risk from ENU exposures during these lifestages, as compared to juvenile exposures.

Table 12. ENU Prenatal and Postnatal ASF_i Mixture Distribution Statistics

Percentiles	Prenatal ASF_i	Postnatal ASF_i
5th	1.59	3.31
10th	2.82	4.78
20th	11.58	8.79
30th	19.68	15.63
40th	34.80	23.46
50th	57.90	37.24
60th	81.39	63.42
70th	348.48	257.98
80th	2038.68	449.21
90th	3798.36	944.82
95th	13144.89	1507.28

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Discussion

Data from studies on 23 unique carcinogens, 20 of which are considered to act via primarily genotoxic modes of action, were analyzed. Of these 20 carcinogens, 15 are thought to require metabolic activation to the ultimate carcinogenic species. The analyses indicate that both the prenatal and postnatal lifestages can be much more susceptible to developing cancer than the adult lifestage. As an index of inherent susceptibility, one that does not account for the longer time early exposures can manifest, an LP ratio was derived. This index compares the carcinogenicity activity when exposures occur early in life compared to older ages, for the same period of time between initial exposure and observation of effect. For the multi-lifestage exposure studies, the median LP ratio for the postnatal period was 4.6 or 7.5, and the upper 95% confidence bound ranged from 123 to 188, depending on the method of combining the ASF distributions underlying studies on the same chemical.

There were few cases of LP ratios less than 1.0 for the postnatal lifestage. These results indicate that in general, for the chemicals studied, there is inherently greater susceptibility during the early postnatal compared to the adult period. The differences between postnatal and adult susceptibility appear more pronounced once the longer period of time that exposed young have to develop tumors is addressed by taking into account time-of-dosing, in calculating the ASF. The median value for the postnatal ASF indicates for the chemicals studied here a 13.5- fold greater contribution to lifetime cancer risk when exposure occurs during this period, compared to the same exposure averaged throughout the adult period; the upper 90th percentile ASF was 211. The DEN and ENU case studies also exhibited substantial sensitivity in the postnatal lifestage, with inherent susceptibility about half an order of magnitude greater than juveniles for DEN, and about an order of magnitude greater than juveniles for ENU, and again greater susceptibility once the longer period of time that exposed young have to develop tumors is taken into account.

Regarding *in utero* exposure, few studies provided data indicative of equal inherent adult and prenatal susceptibility, with an LP ratio of unity. For the multi-lifestage exposure studies, the prenatal LP ratio distributions are roughly bimodal, with LP ratios for several studies significantly greater than unity and several others significantly less than unity (Figure 6). The median LP ratio mixture distribution was 2.5. The median estimate of the prenatal ASF was 2.9.

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Data from studies on 23 unique carcinogens, 20 of which are considered to act via primarily genotoxic modes of action, were analyzed. Of these ... [35]
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and the mean estimate ws 21.1. This modality in the prenatal LP ratio and ASF mixture distributions is reflected in the case studies. The prenatal LP_i ratio for DEN has a median of 0.1, and the majority of the distribution falls below unity. This is suggestive of reduced inherent susceptibility *in utero*. In contrast the median prenatal LP_i ratio for ENU was 19.3, with the majority of the distribution exceeding unity, indicative of greater inherent *in utero* susceptibility. In considering implications of the DEN and ENU case studies it is important to recognize that the referent groups were juvenile rather than adult animals. The prenatal (and postnatal) LP_i ratios and ASF_is are likely to be underestimates, to the extent that some of the apparent sensitivity for DEN and ENU in the early postnatal period carries through to the juvenile period.

ENU is a direct acting carcinogen that does not require metabolic activation to alkylate DNA, forming DNA adducts and mutations that ultimately result in the formation of tumors (Slikker III *et al.,* 2004). In contrast, DEN requires metabolic activation by cytochrome P450 enzymes (e.g., P450 2E1, P450 2A6) to form the active DNA ethylating species (Brittebo *et al.*, 1981). While both ENU and DEN cross the placenta and are widely distributed in fetal tissues (Rice *et al.* 1989; Brittebo *et al.*, 1981), DEN can not be metabolized to any significant extent by fetal tissues until relatively late in gestation (i.e., gestation day 18 in the mouse), and after birth the expression of P450 2E1 progressively increases, reaching adult levels by day 30 (Brittebo *et al.*, 1981). This may explain the lower fetal susceptibility of DEN. However, the multi-lifestage exposure studies illustrate that *in utero* metabolic status is not the sole determinant of *in utero* susceptibility: benzidine and safrole require metabolic activation and exhibit greater susceptibility from prenatal exposure (see Figure 6).

There are just five chemicals and seven studies, two of which are not independent (i.e., the MNU studies of Grubbs *et al*., 1983), available to examine susceptibility in the juvenile lifestage. The LP ratio distributions indicate significantly greater inherent susceptibility in this period for three of the independent studies, with the three remaining independent studies consistent with equal inherent susceptibility to adult animals (Figure 14). For the juvenile lifestage, the ASF mixture distribution was 4.5 at the 50th percentile and 19.7 at the 95th percentile.

The studies that comprise the set of multi-lifestage exposure studies available for these analyses were not homogeneous. That is, they do not represent observations from the same distribution.

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Sensitivity analyses were conducted to test the robustness of the findings to different procedures for analyzing data and combining results. Of the methods used to combine the LP ratio distributions for underlying studies within each lifestage, the method of equally weighting studies within a chemical appears to best represent the available data. The use of inverse variance in weighting LP ratio distributions within a chemical may underweight small studies and overweight large ones, and thus produce a LP ratio mixture distribution that does not accurately reflect the overall data. This is clearly illustrated by the results of the postnatal ENU case study analyses. The method of selecting a single study (i.e., that with the largest median LP ratio) to represent each chemical may also result in inadvertent bias if a selected study is not representative of the group being studied.

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In taking into account the longer period of time for early carcinogen exposures to manifest, the hazard function was assumed to increase with the third power of age. If the true rate of increase with age is greater than that, then the ASFs presented here may result in underestimates of the true sensitivity of these early lifestages.

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As the multi-lifestage exposure and chemical-specific case studies show, there appears to be considerable variability in age-at-exposure related susceptibility across carcinogens. There is also variability in age-at-exposure related susceptibility among studies of the same carcinogen. The sources of variability evident in the analyzed studies include timing of exposure within a given lifestage, and gender, strain, and species differences in tumor response. The set of studies identified and analyzed was not sufficiently robust to fully describe quantitatively the variability. This variability raises concerns that selection of the median (the 50th percentile) estimates may considerably underestimate effects for certain carcinogens or population groups. Relatively large variability in humans in response to carcinogens is expected to be common (Finkel, 1995; 2002).

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Several of the carcinogens studied induced tumors at multiple sites in the same experiment, and at different sites, depending upon the lifestage during which exposure occurred. The cancer potencies used in the early vs. later life comparisons were based on all treatment-related tumors. When treatment-related tumors were induced at multiple sites in the same experiment, or at the same site, but arising from different cell types, the slopes of the dose response curves from these different tumor sites or types were statistically combined to create an overall multisite cancer

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potency distribution for that experiment. The result reflects the total cancer impact associated with the carcinogen exposure in question. This approach differs from other researchers investigating early vs. late in life differences (e.g., Barton *et al.*, 2005; Hattis *et al.*, 2004; 2005). We believe this provides a more complete approach for considering age specific differences in carcinogenic activity.

One limitation of the approach was the focus on lifestages, without attempting to describe changes in susceptibility that occur within a lifestage. Timing of carcinogen exposure within a given lifestage can affect the cancer outcome observed. This is illustrated by experiments with 1-ethyl-1-nitrosobiuret in prenatal and adult rats by Druckrey and Landschutz (1971). A three fold difference in activity was observed between two prenatal exposure groups, one exposed on prenatal day -10 and the other on prenatal day -3 (See Figure 6 and Appendix B, Table B1). The timing of exposure within the adult age window can also affect the cancer outcome, as illustrated by the experiments of Grubbs *et al.* (1983), in which female rats exposed early in the adult period (days 80 through 87) were more than three times as sensitive to the breast cancer effects of MNU than females exposed six weeks later (Figure 14 and Appendix B, Table B3). In general the adult comparison groups in the multi-lifestage exposure studies were fairly young. The extent to which this may result in an overall bias of the results presented here is unclear. Also for several cases, juvenile animals were used as the later life exposure group. In these cases the ASFs are likely underestimates of the relative sensitivity of the prenatal and postnatal lifestages, compared to that of the adult lifestage.

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Excluded from the analysis presented here were early in life studies in which exposure of a given exposure group crossed multiple lifestages. An example of results from studies of this type is provided by mouse studies for two non-genotoxic carcinogens, diphenylhydantoin (Chhabra *et al.*, 1993a) and polybrominated biphenyls (Chhabra *et al.*, 1993ab), in which exposures began prior to conception, and continued throughout the prenatal, postnatal, and post-weaning periods, up to the age of eight weeks. The data, shown in Appendix F, demonstrate an increased sensitivity associated with exposures to either of these non-genotoxic carcinogens during the entire early life period, as compared to exposures during only the adult lifestage. Some studies that crossed multiple lifestages were included in the analyses of Barton *et al.* (2005), which are consistent with the general conclusions here.

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Barton *et al.* (2005) discussed data on 18 unique carcinogens, but ultimately analyzed data on six mutagenic carcinogens (benzidine, diethylnitrosamine, 3-MC, safrole, urethane, and vinyl chloride) to derive the age dependent adjustment factor of 10 for carcinogen exposures occurring between birth and the second birthday, as specified in the U.S. EPA's (U.S. EPA, 2005) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens.* In performing the analysis, Barton *et al.* (2005) compared tumor site-specific potencies, while here multi-site cancer potency estimates provide the basis for comparison. Barton *et al.* (2005) also did not address prenatal or juvenile exposures in their analyses, nor was the issue of time-of dosing addressed wherein exposure to the fetus, infant or child has a longer period of time compared to an exposed adult to produce cancer. Other evaluations of exposure occurring in early life and in adults in the same study have been attempted (e.g., McConnell, 1992) but have not considered indices of carcinogenic activity as systematically as was done in the analyses here or by Barton *et al.* (2005). Thus the analysis presented here adds to the body of evidence on which to consider methods to use in estimating cancer risk when the young are exposed.

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Implications for Cancer Risk Assessment Guidelines

Taken together the results indicate that early lifestages are generally more sensitive to carcinogen exposure than adults, and that cancer risk assessment practices should take increased sensitivity of the young into account. Here the results of these analyses are reflected on in the context of existing state and federal cancer risk assessment guidelines. The degree that such guidelines adequately address carcinogenic exposures to the fetus, infants and children has been a concern of the California State legislature, which mandated the study presented here, as part of the Children's Environmental Health Initiative (AB 2872, Shelly, HSC section 901). This legislation also required OEHHA to review its own and other Cal/EPA, state and federal guidelines to assess methodologies used and establish new methodologies if needed (HSC section 901 [b] and [c]).

U.S. EPA, California and other states now have legal mandates to ensure that regulatory standards are adequately protective of the fetus, infants and children, and have developed or are considering methodologies that explicitly address the young in cancer risk estimation. In California, the Children's Environmental Health Initiative (HSC section 901 [b]) mandates

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OEHHA to ensure that regulatory standards for carcinogens are adequately protective of fetuses, infants and children. In 2001 OEHHA reported on its review of existing guidelines. California has, on occasion, adjusted dose calculations used in estimating cancer potency with a Doll-Armitage analysis to account for variable dosing over time (e.g., early-in-life exposures). This model can be used to address the longer period of time available for cancer to manifest when exposures occur early in life. It does not however address the issue of inherent tissue susceptibility. OEHHA in 2001 concluded that the existing default mathematical models employed for the purpose of estimating excess cancer risk did not adequately address the possibility that risk from early-in-life exposures may differ from that associated with exposures occurring in adulthood. OEHHA further concluded that there was a need for such methodologies to be developed, tested, and validated (Cal/EPA, 2004). Also, under SB 25 (The Children's Environmental Health Protection Act of 1999, Escutia, HSC section 39600 *et seq.*), in re-evaluating cancer potency values under the Air Toxics Hot Spots program, California is required to take into account general or chemical-specific consideration which suggests that children may be especially susceptible to certain carcinogenic effects.

The U.S. EPA *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005) concluded there is evidence of differential susceptibility for mutagenic carcinogens and recommended adjustments to the adult slope factor and its integration with exposure estimates in estimating cancer risk associated with early life exposures. A ten-fold adjustment to the adult slope factor is suggested for exposures to mutagenic carcinogens occurring from birth up to two years of age, and a three-fold adjustment for such exposures occurring from 2 up to 16 years of age. No adjustment was recommended to address the fetus for increased susceptibility or the full lifetime ahead for cancer to be manifest. No adjustment was suggested for non-mutagenic carcinogens (U.S. EPA, 2005), even though there is increasing appreciation that carcinogens often act by multiple mechanisms, including non-mutagenic mechanisms, and that the relative importance of a given mechanism of action may vary with lifestage. Indeed, evidence from human cancers indicates that epigenetic changes, such as alterations in DNA methylation, are often associated with early events in human carcinogenesis (Baylin, 2005). Thus existing U.S. EPA guidance applies to only a subset of carcinogens, and, while addressing exposures to infants and children, does not acknowledge any effect of carcinogen exposures to the fetus.

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OEHHA recognizes the limitations in the data and analyses presented, as discussed above. Still the analyses do provide some guidance on the extent risk may be over- or underestimated by current approaches. The analyses demonstrate the sensitivity of three early lifestages for the carcinogens analyzed here. While there is a great deal of variability across chemicals in the prenatal ASFs, the data indicate that the potency associated with prenatal carcinogen exposure is not zero. A factor of 10 falls roughly at the 70th percentile for the multi-lifestage exposure studies analysis (Table 6). This value could be applied to the potency estimate when calculating lifetime cancer risk in humans arising from carcinogen exposures that occur *in utero*. Alternatively, factors of 50 and 115 fall roughly at the 90th and 95th percentiles, respectively, for the prenatal ASF derived in the multi-lifestage exposure studies analysis.

The U.S. EPA's factor of 10 for postnatal exposures falls between the 40th and 50th percentiles for postnatal studies (Table 7); thus while it is consistent with the data presented, it may result in underestimates of risk for a reasonable fraction of chemicals. Factors of 210 and 350 fall roughly at the 90th and 95th percentiles, respectively, for the postnatal ASF derived in the multi-lifestage exposure studies analysis. The U.S. EPA's factor of 3 for juvenile exposures is consistent with the range of estimates derived from the multi-lifestage exposure studies, although it falls below the median estimate (Table 8). It is acknowledged that there are few data available on which to base an estimate for the juvenile lifestage. A factor of 3 adjusts for the longer time it takes for cancer to manifest, but is unlikely to fully account for inherent differences in susceptibility to cancer, such as occurs in breast tissue of pubescent girls exposed to radiation. Factors of 13 and 20 fall roughly at the 90th and 95th percentiles, respectively, for the juvenile ASF multi-lifestage exposure studies analysis.

Table 15 illustrates the impact of lifestage specific ASFs on lifetime cancer risk. In this example, exposure to the carcinogen is assumed to occur at a constant exposure rate over the entire lifetime. Risk calculations were performed using the mean, 50th, 70th, and 95th percentile ASF values to adjust the adult cancer potency. As shown in Table 15, when increased susceptibility of the fetus, infants, and children is taken into account by applying 50th percentile ASF values, the total lifetime cancer risk is increased two-fold; applying 70th percentile ASF values increases the risk three-fold, applying mean ASF values increases the risk 4.6-fold, and

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applying 95th percentile ASF values increases the risk 16-fold above the risk estimated in the absence of age-specific adjustments to the potency. Table 15 also shows how the application of the U.S. EPA's adjustment factors for the postnatal and juvenile lifestages in calculating total lifetime cancer risk compares with the use of the ASF values derived from the multi-lifestage exposure studies analyzed here. For example, the use of 70th percentile ASF values as adjustments for the prenatal, postnatal, and juvenile lifestages increases the total lifetime cancer risk almost two-fold above the risk estimated using the U.S. EPA's adjustment factors.

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Concluding Remarks

This report indicates the extent risk may be over- or underestimated by current risk assessment approaches. The analyses support the application of weighting factors to address potential increased susceptibility to carcinogen exposures occurring prenatally and during postnatal and juvenile lifestages. The limitations in the data and analyses are recognized and discussed in the report. Limitations can not explain the age specific differences observed.

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Table 15. Comparison of cancer risk estimates[1] for lifetime exposure to 0.0001 mg/kg-d of a carcinogen with potency 1 (mg/kg-d)$^{-1}$ based on different parameters of ASF distributions, or U.S. EPA values.

Lifestage	Years of life exposed	No adjustment		50th percentile		70th percentile		Mean		95th percentile		U.S. EPA (2005)	
		ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	Factor	Risk
In utero	0.75	0	0.0	3	3.2×10^{-6}	10	1.1×10^{-5}	21	2.2×10^{-5}	115	1.2×10^{-4}	0	0.0
Birth to <2 yr	2	1	2.9×10^{-6}	13	3.7×10^{-5}	28	7.9×10^{-5}	79	2.3×10^{-4}	350	1.0×10^{-3}	10	2.9×10^{-5}
2 to <16 yr	14	1	2×10^{-5}	5	1.0×10^{-4}	7	1.4×10^{-4}	7	1.4×10^{-4}	20	4.0×10^{-4}	3	6.0×10^{-5}
16 to 70 yr	55	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}
Total lifetime risk			1.0×10^{-4}		2.2×10^{-4}		3.1×10^{-4}		4.7×10^{-4}		1.6×10^{-3}		1.7×10^{-4}

[1] Risk accrued in age window = potency x ASF x exposure rate x (years exposed/70 years).

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Appendices

A. Default Body Weights for Rats and Mice During the First Six Months of Life

B. Lifestage Potency (LP) Ratios for Multi-Lifestage Exposure Studies

Prenatal LP Ratios

Postnatal LP Ratios

Juvenile LP Ratios

C. Sensitivity Analyses: LP and ASF Mixture Frequency Distributions for Multi-Lifestage Exposure Studies

Prenatal LP Ratio and ASF Mixture Distributions

Postnatal LP Ratio and ASF Mixture Distributions

Juvenile LP Ratio and ASF Mixture Distributions

D. DEN Case Study: Cancer Potency Distributions for DEN Single Lifestage Exposure Experiments and Sensitivity Analyses

E. ENU Case Study: Cancer Potency Distributions for ENU Single Lifestage Exposure Experiments and Sensitivity Analyses

F. Early Life Across-Lifestage Exposure Studies of Two Non-Genotoxic Carcinogens

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Appendix A

Default Body Weights for Rats and Mice During the First Six Months of Life

This appendix describes the approach taken to calculate body weights when needed for dose calculations. For example, doses administered during the postnatal and juvenile lifestages may have been reported as bolus amounts administered (e.g., milligrams) and the publication may not have reported the weight of the animals on the day of compound administration. Because in neonatal and juvenile rodents, body weight changes rapidly through development, default body weights for the first six months of life (i.e., day 1-168) were estimated for each postnatal day for mice and rats, for use in calculating dose in mg/kg-bd wt when body weight on the day of dosing was not reported.

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Growth Model Applied

When standard growth models were applied to the data (e.g., models of Richards, Gompertz, and Janoschek), most seemed to overpredict body weight at very young ages. Thus, OEHHA applied a more flexible model, which was constrained to pass through the actual data point for the day 1 body weight. The modeling was performed using constrained linear regression using the statistical package, STATA (Stata Corp, College Station, Texas). The model takes the form:

$$\text{BodyWeight}_{age} = \beta_0 + \beta_1 (\text{day-1}) + \beta_2 (\text{day-1})^2 + \beta_3 (\text{day-1})^3 + \beta_4 (\text{day-1})^4 \qquad \text{(Eqn. A-1)}$$

where β_0 is defined as the measured average body weight on day 1 of life (i.e., redefining day 1 as 'day 0' or the origin). The variable day is the day of life, and parameters, β_1, β_2, β_3, β_4 are estimated. Fitted values for each day of life through six months of age (i.e., day 168) are provided in look up tables, which are appended.

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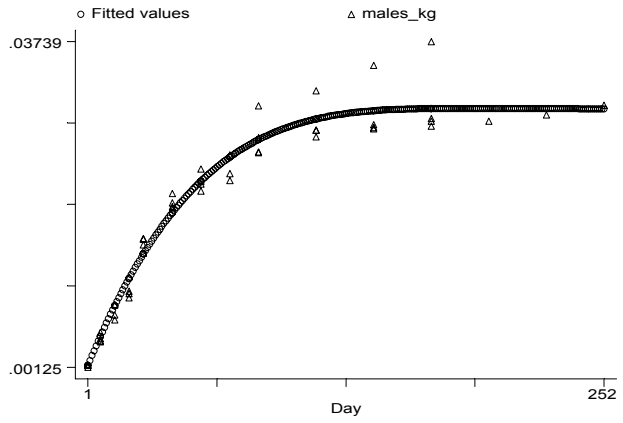
Default body weights were estimated using data from a survey of Poiley (1972) for several strains of mice. Data from BALB/cANCr, AKR/LwCr and C57Bl/6Cr mice were selected for use in deriving the default value, as these datasets comprised the largest numbers of animals surveyed (i.e., early life groups represented averages of 256 to 547 mice for each species). Table A7 gives the data used in the model fitting. Body weights for all three species were quite similar during the first 70 days of life. The AKR/LwCr mice became heavier than the other two species later in life, thus taken together data from these three strains likely provide a reasonable average.

Figure A1 displays the model fit for data from BALB/c, C57Bl/6Cr, AKR/LwCr, and DBA/2Cr mouse strains. Two plots are shown. The first plot shows the data and model fit for male mice, and the second plot does the same for female mice.

Figure A1. Model Fitted Data for Male and Female Mice



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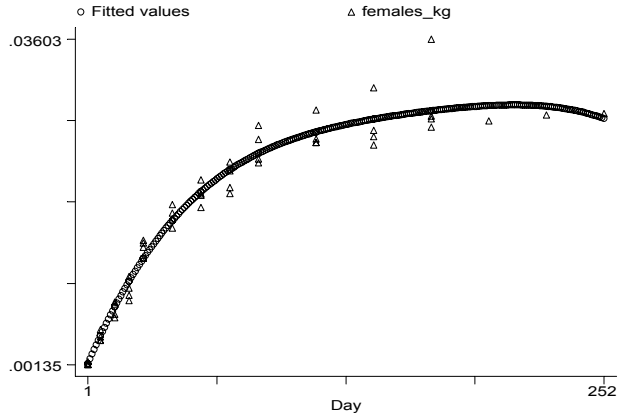

Tables A1 and A2 give the default day-specific body weight values for male and female mice based on these model fits.

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Table A1. Male Mice: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00144	44	0.01894	87	0.02684	130	0.02950
2	0.00199	45	0.01921	88	0.02695	131	0.02953
3	0.00254	46	0.01948	89	0.02705	132	0.02955
4	0.00308	47	0.01974	90	0.02715	133	0.02958
5	0.00361	48	0.02000	91	0.02725	134	0.02960
6	0.00414	49	0.02026	92	0.02735	135	0.02962
7	0.00465	50	0.02050	93	0.02744	136	0.02964
8	0.00516	51	0.02075	94	0.02753	137	0.02966
9	0.00566	52	0.02099	95	0.02762	138	0.02968
10	0.00616	53	0.02122	96	0.02771	139	0.02970
11	0.00664	54	0.02145	97	0.02779	140	0.02972
12	0.00712	55	0.02168	98	0.02787	141	0.02974
13	0.00759	56	0.02190	99	0.02795	142	0.02975
14	0.00806	57	0.02212	100	0.02803	143	0.02977
15	0.00851	58	0.02233	101	0.02811	144	0.02978
16	0.00896	59	0.02254	102	0.02818	145	0.02980
17	0.00940	60	0.02274	103	0.02825	146	0.02981
18	0.00984	61	0.02294	104	0.02832	147	0.02982
19	0.01027	62	0.02313	105	0.02839	148	0.02983
20	0.01069	63	0.02333	106	0.02845	149	0.02984
21	0.01110	64	0.02351	107	0.02851	150	0.02985
22	0.01151	65	0.02370	108	0.02857	151	0.02986
23	0.01191	66	0.02388	109	0.02863	152	0.02987
24	0.01231	67	0.02405	110	0.02869	153	0.02988
25	0.01270	68	0.02422	111	0.02874	154	0.02989
26	0.01308	69	0.02439	112	0.02880	155	0.02990
27	0.01345	70	0.02456	113	0.02885	156	0.02990
28	0.01382	71	0.02472	114	0.02890	157	0.02991
29	0.01419	72	0.02487	115	0.02895	158	0.02992
30	0.01454	73	0.02503	116	0.02900	159	0.02992
31	0.01490	74	0.02518	117	0.02904	160	0.02993
32	0.01524	75	0.02532	118	0.02908	161	0.02993
33	0.01558	76	0.02547	119	0.02913	162	0.02994
34	0.01591	77	0.02561	120	0.02917	163	0.02994
35	0.01624	78	0.02575	121	0.02921	164	0.02994
36	0.01656	79	0.02588	122	0.02924	165	0.02995
37	0.01688	80	0.02601	123	0.02928	166	0.02995
38	0.01719	81	0.02614	124	0.02932	167	0.02995
39	0.01749	82	0.02626	125	0.02935	168	0.02996
40	0.01779	83	0.02638	126	0.02938		
41	0.01809	84	0.02650	127	0.02941		
42	0.01838	85	0.02662	128	0.02944		
43	0.01866	86	0.02673	129	0.02947		

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Table A2. Female Mice: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00147	44	0.01719	87	0.02418	130	0.02710
2	0.00198	45	0.01742	88	0.02428	131	0.02715
3	0.00248	46	0.01766	89	0.02438	132	0.02719
4	0.00298	47	0.01789	90	0.02447	133	0.02723
5	0.00346	48	0.01812	91	0.02457	134	0.02727
6	0.00394	49	0.01834	92	0.02466	135	0.02732
7	0.00441	50	0.01856	93	0.02475	136	0.02736
8	0.00488	51	0.01877	94	0.02484	137	0.02740
9	0.00533	52	0.01898	95	0.02493	138	0.02744
10	0.00578	53	0.01918	96	0.02501	139	0.02748
11	0.00622	54	0.01939	97	0.02509	140	0.02752
12	0.00665	55	0.01958	98	0.02518	141	0.02755
13	0.00708	56	0.01978	99	0.02526	142	0.02759
14	0.00750	57	0.01997	100	0.02533	143	0.02763
15	0.00791	58	0.02015	101	0.02541	144	0.02766
16	0.00832	59	0.02033	102	0.02549	145	0.02770
17	0.00872	60	0.02051	103	0.02556	146	0.02774
18	0.00911	61	0.02069	104	0.02563	147	0.02777
19	0.00949	62	0.02086	105	0.02570	148	0.02781
20	0.00987	63	0.02103	106	0.02577	149	0.02784
21	0.01024	64	0.02119	107	0.02584	150	0.02787
22	0.01061	65	0.02135	108	0.02591	151	0.02791
23	0.01097	66	0.02151	109	0.02597	152	0.02794
24	0.01132	67	0.02167	110	0.02604	153	0.02797
25	0.01167	68	0.02182	111	0.02610	154	0.02800
26	0.01201	69	0.02197	112	0.02616	155	0.02804
27	0.01234	70	0.02211	113	0.02622	156	0.02807
28	0.01267	71	0.02226	114	0.02628	157	0.02810
29	0.01299	72	0.02240	115	0.02634	158	0.02813
30	0.01331	73	0.02253	116	0.02640	159	0.02816
31	0.01362	74	0.02267	117	0.02645	160	0.02819
32	0.01393	75	0.02280	118	0.02651	161	0.02822
33	0.01423	76	0.02293	119	0.02656	162	0.02825
34	0.01452	77	0.02305	120	0.02662	163	0.02827
35	0.01481	78	0.02318	121	0.02667	164	0.02830
36	0.01509	79	0.02330	122	0.02672	165	0.02833
37	0.01537	80	0.02342	123	0.02677	166	0.02836
38	0.01565	81	0.02353	124	0.02682	167	0.02838
39	0.01592	82	0.02365	125	0.02687	168	0.02841
40	0.01618	83	0.02376	126	0.02692		
41	0.01644	84	0.02387	127	0.02696		
42	0.01669	85	0.02397	128	0.02701		
43	0.01694	86	0.02408	129	0.02706		

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Default body weights applicable to all rat strains except Sprague-Dawley rats were estimated using data from surveys by Poiley (1972) and Cameron *et al.* (1985) for Fischer 344 (F344) rats (See Table A8). The body weights of F344 rats are reasonably representative of most other rat strains (U.S. EPA, 1988). Data from Sprague-Dawley rats, which become much heavier than most other rat strains, were used to estimate default body weights for this strain using normative data surveyed by Poiley (1972) (See Table A9). Figure A2 displays the model fit for data from the F344 rat strain. The first plot shows the fit for males, the second for females. Figure A3 displays the model fit for data from the Sprague-Dawley rat strain. The first plot shows the fit for males, the second for females.

Figure A2. Model Fitted Data for Male and Female F344 Rats

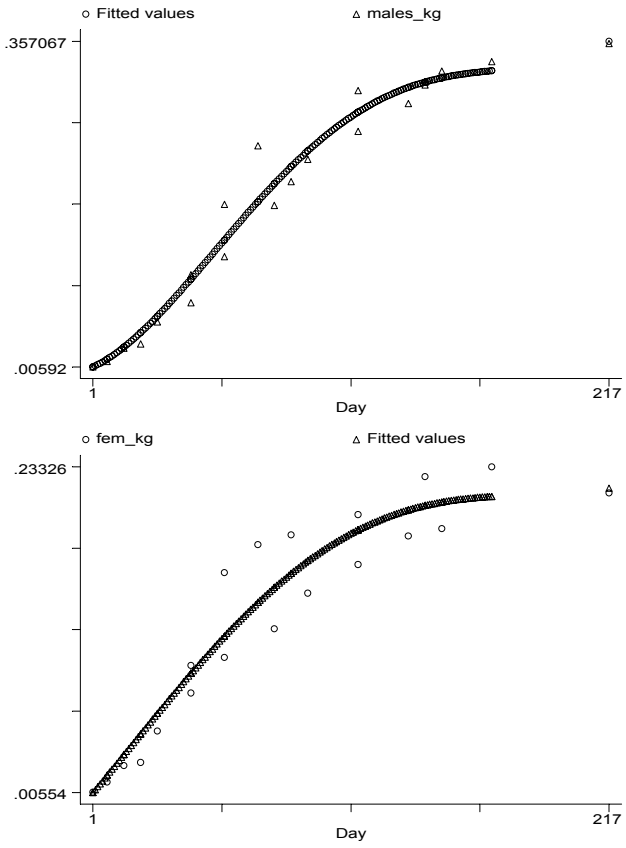

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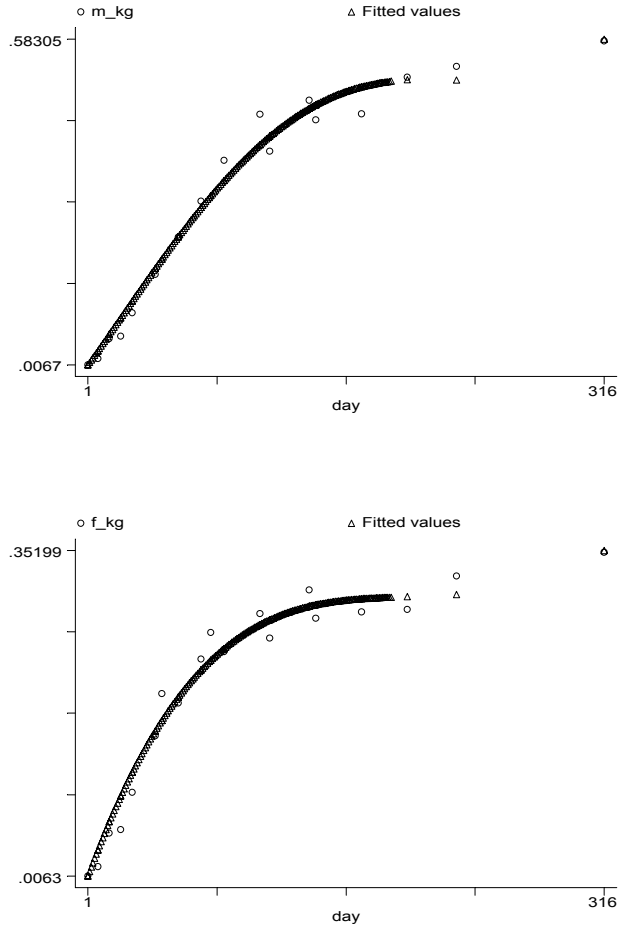
Figure A3. Model Fitted Data for Male and Female Sprague-Dawley Rats



Tables A3 and A4 give the default day-specific body weight values for male and female rats (with the exception of Sprague-Dawley rats) based on these model fits. The default day-specific body weight values for male and female Sprague-Dawley rats were based on model fits derived from data specific to Sprague-Dawley rats. These values are shown in Tables A5 and A6.

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Table A3. Male Rats: Default Body Weight for the First 168 Days of Life (based on F344 Rats; default does not apply to Sprague-Dawley rats)

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00592	44	0.10643	87	0.22932	130	0.30443
2	0.00712	45	0.10943	88	0.23176	131	0.30544
3	0.00839	46	0.11244	89	0.23416	132	0.30641
4	0.00975	47	0.11545	90	0.23654	133	0.30736
5	0.01118	48	0.11847	91	0.23888	134	0.30828
6	0.01268	49	0.12150	92	0.24120	135	0.30916
7	0.01426	50	0.12452	93	0.24348	136	0.31002
8	0.01591	51	0.12755	94	0.24573	137	0.31085
9	0.01762	52	0.13058	95	0.24795	138	0.31165
10	0.01940	53	0.13361	96	0.25014	139	0.31242
11	0.02125	54	0.13664	97	0.25230	140	0.31316
12	0.02315	55	0.13966	98	0.25442	141	0.31387
13	0.02512	56	0.14268	99	0.25651	142	0.31456
14	0.02714	57	0.14570	100	0.25857	143	0.31523
15	0.02923	58	0.14871	101	0.26059	144	0.31586
16	0.03136	59	0.15171	102	0.26258	145	0.31648
17	0.03355	60	0.15471	103	0.26454	146	0.31706
18	0.03579	61	0.15769	104	0.26646	147	0.31763
19	0.03808	62	0.16067	105	0.26835	148	0.31817
20	0.04042	63	0.16363	106	0.27021	149	0.31869
21	0.04280	64	0.16658	107	0.27203	150	0.31919
22	0.04523	65	0.16952	108	0.27382	151	0.31966
23	0.04769	66	0.17245	109	0.27557	152	0.32012
24	0.05020	67	0.17536	110	0.27728	153	0.32056
25	0.05275	68	0.17826	111	0.27897	154	0.32098
26	0.05533	69	0.18114	112	0.28061	155	0.32138
27	0.05796	70	0.18400	113	0.28223	156	0.32176
28	0.06061	71	0.18684	114	0.28381	157	0.32213
29	0.06330	72	0.18967	115	0.28535	158	0.32248
30	0.06601	73	0.19247	116	0.28686	159	0.32282
31	0.06876	74	0.19526	117	0.28833	160	0.32314
32	0.07153	75	0.19802	118	0.28977	161	0.32345
33	0.07433	76	0.20077	119	0.29118	162	0.32375
34	0.07716	77	0.20349	120	0.29255	163	0.32404
35	0.08000	78	0.20619	121	0.29389	164	0.32432
36	0.08287	79	0.20886	122	0.29519	165	0.32458
37	0.08576	80	0.21151	123	0.29646	166	0.32485
38	0.08867	81	0.21413	124	0.29770	167	0.32510
39	0.09159	82	0.21673	125	0.29890	168	0.32535
40	0.09454	83	0.21930	126	0.30007		
41	0.09749	84	0.22185	127	0.30121		
42	0.10046	85	0.22437	128	0.30231		
43	0.10344	86	0.22686	129	0.30339		

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Table A4. Female Rats: Default Body Weight for the First 168 Days of Life (based on F344 Rats; default does not apply to Sprague-Dawley rats)

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00554	44	0.09273	87	0.16280	130	0.20171
2	0.00756	45	0.09465	88	0.16408	131	0.20224
3	0.00959	46	0.09655	89	0.16534	132	0.20276
4	0.01162	47	0.09844	90	0.16658	133	0.20325
5	0.01365	48	0.10032	91	0.16780	134	0.20374
6	0.01570	49	0.10219	92	0.16901	135	0.20420
7	0.01774	50	0.10405	93	0.17020	136	0.20466
8	0.01980	51	0.10589	94	0.17137	137	0.20509
9	0.02185	52	0.10772	95	0.17253	138	0.20552
10	0.02391	53	0.10955	96	0.17366	139	0.20593
11	0.02597	54	0.11136	97	0.17478	140	0.20632
12	0.02803	55	0.11315	98	0.17588	141	0.20670
13	0.03010	56	0.11494	99	0.17696	142	0.20707
14	0.03216	57	0.11671	100	0.17802	143	0.20743
15	0.03423	58	0.11846	101	0.17907	144	0.20777
16	0.03630	59	0.12021	102	0.18010	145	0.20810
17	0.03836	60	0.12194	103	0.18111	146	0.20841
18	0.04043	61	0.12365	104	0.18210	147	0.20871
19	0.04250	62	0.12535	105	0.18307	148	0.20900
20	0.04456	63	0.12704	106	0.18403	149	0.20928
21	0.04662	64	0.12871	107	0.18496	150	0.20955
22	0.04869	65	0.13037	108	0.18588	151	0.20980
23	0.05074	66	0.13202	109	0.18679	152	0.21005
24	0.05280	67	0.13364	110	0.18767	153	0.21028
25	0.05485	68	0.13526	111	0.18853	154	0.21050
26	0.05690	69	0.13685	112	0.18938	155	0.21071
27	0.05894	70	0.13843	113	0.19021	156	0.21091
28	0.06098	71	0.14000	114	0.19103	157	0.21111
29	0.06302	72	0.14155	115	0.19182	158	0.21129
30	0.06505	73	0.14308	116	0.19260	159	0.21146
31	0.06707	74	0.14460	117	0.19336	160	0.21162
32	0.06909	75	0.14610	118	0.19410	161	0.21178
33	0.07111	76	0.14759	119	0.19483	162	0.21192
34	0.07311	77	0.14905	120	0.19554	163	0.21206
35	0.07511	78	0.15051	121	0.19623	164	0.21219
36	0.07710	79	0.15194	122	0.19691	165	0.21232
37	0.07909	80	0.15336	123	0.19756	166	0.21243
38	0.08106	81	0.15476	124	0.19821	167	0.21254
39	0.08303	82	0.15614	125	0.19883	168	0.21264
40	0.08499	83	0.15751	126	0.19944		
41	0.08694	84	0.15886	127	0.20003		
42	0.08888	85	0.16019	128	0.20061		
43	0.09081	86	0.16150	129	0.20117		

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Table A5. Male Sprague-Dawley Rats: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00670	44	0.17648	87	0.35543	130	0.42990
2	0.00877	45	0.18129	88	0.35848	131	0.43110
3	0.01099	46	0.18611	89	0.36149	132	0.42985
4	0.01334	47	0.19091	90	0.36425	133	0.43218
5	0.01583	48	0.19570	91	0.36715	134	0.43204
6	0.01845	49	0.20048	92	0.37000	135	0.43144
7	0.02120	50	0.20525	93	0.37257	136	0.43240
8	0.02406	51	0.21000	94	0.37529	137	0.43290
9	0.02705	52	0.21473	95	0.37792	138	0.43292
10	0.03015	53	0.21944	96	0.38048	139	0.43448
11	0.03336	54	0.22413	97	0.38276	140	0.43355
12	0.03668	55	0.22880	98	0.38515	141	0.43415
13	0.04009	56	0.23343	99	0.38745	142	0.43426
14	0.04361	57	0.23804	100	0.38986	143	0.43590
15	0.04721	58	0.24271	101	0.39196	144	0.43502
16	0.05091	59	0.24713	102	0.39396	145	0.43565
17	0.05469	60	0.25164	103	0.39565	146	0.43578
18	0.05856	61	0.25623	104	0.39903	147	0.43540
19	0.06250	62	0.26049	105	0.40008	148	0.43651
20	0.06652	63	0.26503	106	0.40283	149	0.43711
21	0.07061	64	0.26944	107	0.40322	150	0.43718
22	0.07476	65	0.27370	108	0.40530	151	0.43672
23	0.07898	66	0.27803	109	0.40704	152	0.43774
24	0.08326	67	0.28221	110	0.40844	153	0.43823
25	0.08760	68	0.28644	111	0.40949	154	0.43817
26	0.09198	69	0.29051	112	0.41220	155	0.43756
27	0.09642	70	0.29462	113	0.41254	156	0.43842
28	0.10091	71	0.29856	114	0.41454	157	0.43872
29	0.10544	72	0.30254	115	0.41616	158	0.44047
30	0.11001	73	0.30655	116	0.41742	159	0.43964
31	0.11461	74	0.31037	117	0.41831	160	0.44026
32	0.11925	75	0.31422	118	0.41881	161	0.44030
33	0.12392	76	0.31787	119	0.42095	162	0.44178
34	0.12862	77	0.32173	120	0.42270	163	0.44268
35	0.13334	78	0.32540	121	0.42203	164	0.44298
36	0.13808	79	0.32887	122	0.42299	165	0.44270
37	0.14285	80	0.33253	123	0.42556	166	0.44383
38	0.14762	81	0.33598	124	0.42571	167	0.44435
39	0.15242	82	0.33922	125	0.42545	168	0.44629
40	0.15722	83	0.34263	126	0.42678		
41	0.16203	84	0.34603	127	0.42770		
42	0.16684	85	0.34920	128	0.42819		
43	0.17166	86	0.35233	129	0.42825		

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Table A6. Female Sprague-Dawley Rats: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00630	44	0.15144	87	0.25154	130	0.28142
2	0.00966	45	0.15449	88	0.25302	131	0.28167
3	0.01304	46	0.15752	89	0.25446	132	0.28101
4	0.01643	47	0.16051	90	0.25580	133	0.28167
5	0.01984	48	0.16348	91	0.25717	134	0.28140
6	0.02326	49	0.16642	92	0.25850	135	0.28097
7	0.02670	50	0.16933	93	0.25972	136	0.28109
8	0.03015	51	0.17221	94	0.26098	137	0.28104
9	0.03360	52	0.17505	95	0.26219	138	0.28081
10	0.03706	53	0.17787	96	0.26336	139	0.28114
11	0.04054	54	0.18065	97	0.26440	140	0.28054
12	0.04401	55	0.18339	98	0.26548	141	0.28050
13	0.04749	56	0.18611	99	0.26650	142	0.28028
14	0.05098	57	0.18879	100	0.26755	143	0.28061
15	0.05446	58	0.19146	101	0.26848	144	0.28001
16	0.05795	59	0.19403	102	0.26935	145	0.27996
17	0.06144	60	0.19660	103	0.27009	146	0.27972
18	0.06492	61	0.19918	104	0.27144	147	0.27928
19	0.06840	62	0.20162	105	0.27192	148	0.27940
20	0.07188	63	0.20414	106	0.27301	149	0.27931
21	0.07535	64	0.20658	107	0.27323	150	0.27903
22	0.07882	65	0.20896	108	0.27405	151	0.27855
23	0.08228	66	0.21133	109	0.27473	152	0.27861
24	0.08573	67	0.21362	110	0.27527	153	0.27847
25	0.08917	68	0.21592	111	0.27567	154	0.27813
26	0.09260	69	0.21813	112	0.27668	155	0.27758
27	0.09602	70	0.22034	113	0.27680	156	0.27757
28	0.09942	71	0.22247	114	0.27751	157	0.27735
29	0.10281	72	0.22458	115	0.27808	158	0.27766
30	0.10619	73	0.22669	116	0.27850	159	0.27702
31	0.10955	74	0.22871	117	0.27877	160	0.27690
32	0.11290	75	0.23072	118	0.27889	161	0.27658
33	0.11623	76	0.23264	119	0.27960	162	0.27678
34	0.11954	77	0.23462	120	0.28016	163	0.27676
35	0.12283	78	0.23650	121	0.27982	164	0.27653
36	0.12610	79	0.23829	122	0.28007	165	0.27608
37	0.12935	80	0.24014	123	0.28090	166	0.27614
38	0.13257	81	0.24189	124	0.28082	167	0.27599
39	0.13578	82	0.24354	125	0.28059	168	0.27635
40	0.13896	83	0.24524	126	0.28094		
41	0.14212	84	0.24691	127	0.28112		
42	0.14525	85	0.24849	128	0.28114		
43	0.14836	86	0.25003	129	0.28099		

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Table A7. Mouse Data Used in Fitting Eqn. 1 (Source: Poiley, 1972)

Strain	Day	Bodyweight (kg)		Strain	Day	Bodyweight (kg)	
		Males	Females			Males	Females
Balb/C	1	0.00125	0.00169	DBA/2Cr	1	0.00148	0.00135
	7	0.00511	0.00509		7	0.00406	0.00396
	14	0.00817	0.00803		14	0.00823	0.00781
	21	0.01127	0.01076		21	0.00969	0.00952
	28	0.01545	0.01432		28	0.01554	0.01462
	42	0.01948	0.0169		42	0.01908	0.01752
	56	0.02082	0.01941		56	0.02188	0.01958
	70	0.02197	0.02022		70	0.02481	0.02202
	84	0.02516	0.02287		84	0.02672	0.02535
	112	0.0276	0.02504		112	0.02682	0.02541
	140	0.02816	0.02476		140	0.02788	0.02565
	168	0.02857	0.02667		168	0.02886	0.02754
	196	0.02857	0.02735				
	224	0.02925	0.02798				
	252	0.03033	0.0281				
AKR/LwCr	1	0.00153	0.00143				
	7	0.00444	0.0043				
	14	0.00704	0.00674				
	21	0.00896	0.00874				
	28	0.01391	0.0127				
	42	0.02053	0.01841				
	56	0.02327	0.02105				
	70	0.02481	0.02296				
	84	0.03028	0.02686				
	112	0.03193	0.02848				
	140	0.03477	0.03087				
	168	0.03739	0.03603				
C57Bl/6Cr	1	0.00149	0.0014				
	7	0.00419	0.00394				
	14	0.00653	0.00637				
	21	0.00941	0.00818				
	28	0.01486	0.01389				
	42	0.01893	0.01592				
	56	0.02159	0.01812				
	70	0.02276	0.0196				
	84	0.02509	0.02328				
	112	0.02756	0.02503				
	140	0.02771	0.02632				
	168	0.02803	0.02787				

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Table A8. F344 Rat Data Used in Fitting Eqn. 1

Age (in days)	Bodyweight (kg)		Reference
	Males	Females	
1	0.00592	0.00574	Poiley, 1972
7	0.01201	0.01285	
14	0.02634	0.02436	
21	0.0307	0.02658	
28	0.05423	0.04849	
42	0.10506	0.09446	
56	0.18112	0.15936	
70	0.24446	0.17893	
84	0.20588	0.18567	
112	0.3042	0.19976	
140	0.31301	0.22657	
168	0.33542	0.23326	
42	0.075	0.075	Cameron *et al*., 1985
56	0.125	0.1	
77	0.18	0.12	
91	0.23	0.145	
112	0.26	0.165	
133	0.29	0.185	
140	0.31	--	
147	0.325	0.19	
217	0.355	0.215	

Table A9. Sprague-Dawley Rat Data Used in Fitting Eqn. 1 (Source: Poiley, 1972)

Age (in days)	Bodyweight (kg)	
	Males	Females
1	0.0067	0.0063
7	0.018	0.0164
14	0.053	0.052
21	0.057	0.0556
28	0.0985	0.0953
42	0.1668	0.1553
56	0.2326	0.1901
70	0.2965	0.2361
84	0.3686	0.2446
112	0.3849	0.259
140	0.4403	0.2803
168	0.4511	0.2868
196	0.5157	0.895

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References

Cameron TP, Hickman RL, Kornreich MR, Tarone RE (1985). History, survival, and growth patterns of B6C3F1 mice and F344 rats in the National Cancer Institute Carcinogenesis Testing Program. *Fundam Appl Toxicol* **5**(3):526-38.

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Appendix B

Lifestage Potency (LP) Ratios for Multi-Lifestage Exposure Studies

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Lifestage cancer potency (LP) ratio distribution statistics derived from multi-lifestage exposure study datasets are presented here. Multi-lifestage exposure studies have at least two groups of animals exposed to a given chemical carcinogen during different lifestages. One dose group is exposed to a chemical only during one early lifestage (either the prenatal, postnatal, or juvenile lifestage). The second dose group is exposed for some period of time at an older age, preferably during the adult lifestage. For each multi-lifestage exposure study, the LP ratio distribution was computed as the quotient of the cancer potency distribution for those animals exposed during the early lifestage (e.g., prenatal, postnatal, or juvenile) and those exposed in later life (e.g., adult, or juvenile in cases where no adult exposure group was included).

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Table B1 presents the prenatal LP ratio distributions and study details for the multi-lifestage exposure datasets that included a prenatal exposure group, grouped by carcinogen. Table B2 presents the postnatal LP ratio distributions and study details for the multi-lifestage exposure datasets that included a postnatal exposure group, grouped by carcinogen. Table B3 presents the juvenile LP ratio distributions and study details for the multi-lifestage exposure datasets that included a juvenile exposure group as the "early life" exposure, grouped by carcinogen.

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Table B1. Multi-Lifestage Exposure Studies: Prenatal Lifestage Potency (LP) Ratios for Different Chemicals

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Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Benzidine	Vesselinovitch *et al.* (1979a)	Mouse*	B6C3F$_1$	Female	No	2	9.12E-01	0.000%	1.36E-01	4.52E-01	7.70E-01	1.22E+00	2.17E+00
		Mouse*	B6C3F$_1$	Male	No	2	4.64E+01	0.000%	2.57E+01	3.54E+01	4.42E+01	5.49E+01	7.46E+01
Butylnitrosourea	Zeller *et al.* (1978)	Rat*	Sprague Dawley	Male/ Female	Yes	2	5.82E-01	0.000%	2.18E-01	3.74E-01	5.30E-01	7.30E-01	1.12E+00
Diethylstilbesterol (DES)	Turusov *et al.* (1992)	Mouse	CBA	Female	No	2	4.07E-01	0.000%	1.38E-01	2.54E-01	3.59E-01	5.02E-01	8.25E-01
Diethylnitrosamine (DEN)	Mohr *et al.* (1975)	Hamster	Syrian Golden	Female	No	2	1.94E+00	0.000%	1.01E+00	1.41E+00	1.80E+00	2.32E+00	3.34E+00
	Mohr *et al.* (1995)	Hamster	Syrian Golden	Female	No	2	5.01E-01	0.000%	2.86E-01	3.87E-01	4.78E-01	5.89E-01	7.95E-01
Dimethylnitrosamine (DMN)	Althoff *et al.* (1977)	Hamster	Syrian Golden	Male/ Female	Yes	2	7.84E+00	4.028%	2.40E-01	4.38E-01	6.86E-01	1.20E+00	1.64E+01
Di-n-propyl-nitrosamine (DPN)	Althoff *et al.* (1977)	Hamster	Syrian Golden	Male/ Female	Yes	2	1.47E-01	0.000%	6.40E-02	1.00E-01	1.34E-01	1.79E-01	2.76E-01
	Althoff and Grandjean (1979)	Hamster	Syrian Golden	Female	No	2	1.18E-01	0.000%	4.03E-02	7.55E-02	1.07E-01	1.49E-01	2.33E-01
1-Ethylnitrosobiuret	Druckrey and Landschutz (1971)	Rat	BD IX	Male/ Female	Yes	2	1.64E+01	0.000%	8.70E+00	1.19E+01	1.51E+01	1.94E+01	2.88E+01
					Yes	2	4.87E+00	0.000%	2.88E+00	3.78E+00	4.62E+00	5.68E+00	7.75E+00

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Table B1. Continued. Prenatal LP Ratios

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Ethylnitrosourea (ENU)	Naito *et al.* (1981)	Rat*	Wistar	Female	No	2	3.28E+01	4.051%	6.55E+00	1.24E+01	2.03E+01	3.56E+01	2.66E+02
				Male	No	2	7.50E+00	0.000%	3.18E+00	4.84E+00	6.62E+00	9.14E+00	1.48E+01
	Tomatis *et al.* (1977)	Rat	BDVI	Female	No	2	2.89E+00	0.000%	1.20E+00	1.85E+00	2.54E+00	3.53E+00	5.76E+00
2-Hydroxypropyl-nitrosamine	Althoff and Grandjean (1979)	Hamster	Syrian Golden	Male/ Female	No	2	1.55E-01	0.000%	2.95E-02	8.34E-02	1.33E-01	2.00E-01	3.54E-01
3-Methyl-cholanthrene (3-MC)	Tomatis *et al.* (1971)	Mouse	CF-1	Female	Yes	2	6.49E-01	0.000%	4.20E-01	5.30E-01	6.26E-01	7.42E-01	9.53E-01
	Turusov *et al.* (1973)	Mouse	CF-1	Female	No	2	4.17E+00	0.000%	2.03E+00	2.92E+00	3.80E+00	5.01E+00	7.54E+00
4-(Methylnitros-amino)-1-(3-pyridyl)-1-butanone (NNK)	Anderson *et al.* (1989)	Mouse	C3H & $B6C3F_1$[a]	Male/ Female[b]	Yes	2	1.66E-01	0.000%	6.18E-02	1.12E-01	1.56E-01	2.09E-01	3.06E-01
Safrole	Vesselinovitch *et al.* (1979a)	Mouse*	$B6C3F_1$	Male	No	2	5.56E+01	1.485%	4.86E+00	1.92E+01	3.51E+01	6.32E+01	1.91E+02
	Vesselinovitch *et al.* (1979b)	Mouse*	$B6C3F_1$	Female	Yes	2	3.37E+00	0.000%	1.12E+00	2.07E+00	3.03E+01	4.31E+00	6.81E+00
Urethane	Choudari Kommineni *et al.* (1970)	Rat*	MRC	Male/ Female	No	2	4.98E+00	1.031%	4.89E-01	1.80E+00	3.31E+00	5.91E+00	1.55E+01
Vinyl chloride	Maltoni *et al.* (1981)	Rat	Sprague Dawley	Male/ Female	Yes	2	2.57E+00	0.000%	1.28E+00	1.92E+00	2.46E+00	3.10E+00	4.19E+00

* Later life exposure group was dosed during the later part of the juvenile period.
[a] Pregnant C3H females were mated with C57BL males to produce $B6C3F_1$ offspring.
[b] C3H adult females; $B6C3F_1$ prenatal males.

Table B2. Multi-Lifestage Exposure Studies: Postnatal Lifestage Potency (LP) Ratios for Different Chemicals

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Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Benzidine	Vesselinovitch *et al.* (1975b)	Mouse*	$B6C3F_1$	Male	No	2	9.98E+01	0.000%	6.75E+01	8.23E+01	9.54E+01	1.12E+02	1.46E+02
	Vesselinovitch *et al.* (1979)	Mouse*	$B6C3F_1$	Female	No	2	8.76E-01	0.000%	1.66E-01	4.34E-01	7.39E-01	1.17E+00	2.07E+00
				Male	No	2	1.95E+02	0.000%	1.21E+02	1.56E+02	1.88E+02	2.26E+02	2.98E+02
Benzo[a]pyrene	Truhaut *et al.* (1966)	Mouse*	Swiss	Male/ Female	No	2	6.20E-01	0.000%	2.55E-01	3.88E-01	5.31E-01	7.43E-01	1.28E+00
	Vesselinovitch *et al.* (1975a)	Mouse*	$B6C3F_1$	Female	Yes	2	2.28E+00	0.000%	1.50E+00	1.86E+00	2.18E+00	2.60E+00	3.42E+00
				Male	Yes	2 & 3[c]	1.96E+00	0.000%	1.42E+00	1.70E+00	1.93E+00	2.18E+00	2.61E+00
			$C3AF_1$	Female	Yes	2	1.90E+00	0.000%	1.14E+00	1.50E+00	1.82E+00	2.21E+00	2.94E+00
				Male	Yes	2	2.06E+00	0.000%	1.20E+00	1.59E+00	1.94E+00	2.40E+00	3.30E+00
1,1-Bis(*p*-Chlorophenol)-2,2,2-trichloroethane (DDT)	Vesselinovitch *et al.* (1979a)	Mouse*	$B6C3F_1$	Male	No	2	1.46E+01	0.000%	8.43E-01	5.61E+00	9.68E+00	1.56E+01	3.25E+01
Butylnitrosourea	Zeller *et al.* (1978)	Rat*	Sprague Dawley	Male/ Female	Yes	2	3.99E+00	0.000%	2.46E+00	3.17E+00	3.80E+00	4.60E+00	6.14E+00
Dibutylnitrosamine	Wood *et al.* (1970)	Mouse	IF x C57	Female	Yes	2	7.49E+01	0.000%	3.32E+01	4.96E+01	6.64E+01	9.04E+01	1.45E+02
				Male	Yes	2	8.04E+01	0.000%	3.53E+01	5.25E+01	7.08E+01	9.73E+01	1.59E+02

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Table B2. Continued. Postnatal LP Ratios

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Diethylnitrosamine (DEN)	Rao and Vesselinovitch (1973)	Mouse*	$B6C3F_1$	Male	No	2	2.8E+01	0.000%	1.25E+01	1.84E+01	2.47E+01	3.37E+01	5.45E+01
	Vesselinovitch *et al.* (1984)	Mouse*	$B6C3F_1$	Female (day 1)[a]	Yes	2 & 3[c]	2.28E+00	0.000%	1.57E+00	1.92E+00	2.22E+00	2.57E+00	3.20E+00
				Male (day 1)[a]	Yes	2 & 3[c]	5.23E+00	0.000%	3.67E+00	4.46E+00	5.12E+00	5.88E+00	7.18E+00
				Female (day 15)[b]	Yes	2 & 3[c]	1.75E+00	0.000%	1.20E+00	1.47E+00	1.71E+00	1.98E+00	2.47E+00
				Male (day 15)[b]	Yes	2 & 3[c]	4.50E+00	0.000%	3.22E+00	3.87E+00	4.41E+00	5.03E+00	6.10E+00
			$C3AF_1$	Female (day 1)[a]	Yes	2	1.27E+00	0.000%	6.60E-01	9.40E-01	1.20E+00	1.52E+00	2.15E+00
				Male (day 1)[a]	Yes	2	2.90E+00	0.000%	1.75E+00	2.30E+00	2.79E+00	3.37E+00	4.42E+00
				Female (day 15)[b]	Yes	2	6.00E-01	0.000%	3.10E-01	4.50E-01	5.70E-01	7.20E-01	1.01E+00
				Male (day 15)[b]	Yes	2	1.69+00	0.000%	1.01E+00	1.34E+00	1.62E+00	1.97E+00	2.59E+00
7,12-Dimethyl-benz[a]anthracene (DMBA)	Meranze *et al.* (1969)	Rat	Fels-Wistar	Female	Yes	2	2.24E+01	0.248%	6.89E+00	1.03E+01	1.44E+01	2.12E+01	4.68E+01
				Male	Yes	2	1.59E+01	0.000%	6.03E+00	9.61E+00	1.35E+01	1.93E+01	3.37E+01
	Walters (1966)	Mouse	BALB/c	Female	No	2	1.30E+00	0.000%	6.78E-01	9.59E-01	1.22E+00	1.55E+00	2.20E+00
				Male	No	2	6.96E-01	0.000%	3.21E-01	4.81E-01	6.39E-01	8.46E-01	1.27E+00
1,2-Dimethylhydrazine	Martin *et al.* (1974)	Rat*	BDIX	Male/ Female	No	2	2.47E-01	0.000%	6.33E-02	1.31E-01	2.05E-01	3.15E-01	5.71E-01

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Table B2. Continued. Postnatal LP Ratios

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
1-Ethylnitrosobiuret	Druckrey and Landschutz (1971)	Rat	BD IX	Male/ Female	Yes	2	1.34E+01	0.000%	6.13E+00	9.31E+00	1.24E+01	1.63E+01	2.42E+01
Ethylnitrosourea (ENU)	Naito *et al.* (1985)	Gerbil*	Mongolian	Female	No	2	7.64E-01	0.000%	1.53E-01	3.60E-01	5.94E-01	9.66E-01	1.91E+00
				Male	No	2	4.37E+00	2.975%	8.20E-01	1.61E+00	2.70E+00	4.78E+00	1.97E+01
	Bosch (1977)	Rat*	WAG	Female	Yes	2	5.03E+00	0.000%	1.80E+00	2.98E+00	4.28E+00	6.20E+00	1.08E+01
				Male	Yes	2	3.51E+00	0.000%	1.07E+00	1.85E+00	2.78E+00	4.29E+00	8.40E+00
	Naito *et al.* (1981)	Rat*	Wistar	Female	Yes	2	2.28E+01	4.051%	5.30E+00	9.24E+00	1.46E+01	2.46E+01	1.87E+02
				Male	Yes	2	2.82E+00	0.000%	1.35E+00	1.94E+00	2.55E+00	3.40E+00	5.20E+00
	Vesselinovitch *et al.* (1974)	Mouse*	$B6C3F_1$	Female (day 1)[a]	Yes	2	1.98E+00	0.000%	1.32E+00	1.64E+00	1.91E+00	2.25E+00	2.88E+00
				Male (day 1)[a]	Yes	2	1.80E+00	0.000%	1.35E+00	1.59E+00	1.77E+00	1.98E+00	2.33E+00
				Female (day 15)[b]	Yes	2	1.22E+00	0.000%	9.09E-01	1.07E+00	1.20E+00	1.35E+00	1.59E+00
				Male (day 15)[b]	Yes	2	2.65E+00	0.000%	1.89E+00	2.27E+00	2.59E+00	2.96E+00	3.64E+00
			$C3AF_1$	Female	Yes	2	2.94E+00	0.000%	1.93E+00	2.39E+00	2.81E+00	3.33E+00	4.41E+00
				Male (day 1)[a]	Yes	2	6.95E+00	0.000%	4.32E+00	5.55E+00	6.65E+00	8.01E+00	1.06E+01
				Male (day 15)[b]	Yes	2	4.90E+00	0.000%	3.19E+00	4.01E+00	4.72E+00	5.59E+00	7.23E+00
3-Hydroxyxanthine	Anderson *et al.* (1978)	Rat	Wistar	Female	No	2	8.15E+00	1.551%	0.00E+00	2.19E+00	4.60E+00	8.77E+00	2.95E+01
3-Methyl-cholanthrene (3-MC)	Klein (1959)	Mouse	A/He	Female	Yes	2	4.58E+00	0.000%	2.14E+00	3.14E+00	4.15E+00	5.51E+00	8.50E+00
				Male	Yes	2	5.48E+00	0.000%	2.95E+00	4.06E+00	5.12E+00	6.50E+00	9.26E+00

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Table B2. Continued. Postnatal LP Ratios

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Methylnitrosourea (MNU)	Terracini and Testa (1970)	Mouse*	$B6C3F_1$	Female	Yes	2	1.29E+00	0.000%	7.87E-01	1.03E+00	1.24E+00	1.49E+00	1.96E+00
				Male	Yes	2	3.36E+00	0.000%	2.07E+00	2.69E+00	3.23E+00	3.88E+00	5.07E+00
	Terracini *et al.* (1976)	Mouse	C3Hf/Dp	Female	Yes	2	1.07E+00	0.000%	5.90E-01	8.28E-01	1.03E+00	1.26E+00	1.69E+00
				Male	Yes	2	8.21E-01	0.000%	5.48E-01	6.83E-01	7.96E-01	9.32E-01	1.18E+00
β-Propiolactone	Chernozemski and Warwick (1970)	Mouse	$B6AF_1$	Female	No	2	1.29E+00	0.525%	3.38E-01	6.38E-01	9.77E-01	1.52E+00	3.13E+00
				Male	No	2	1.07E+01	0.983%	2.39E+00	4.01E+00	5.97E+00	9.27E+00	2.14E+01
Safrole	Vesselinovitch *et al.* (1979a)	Mouse*	$B6C3F_1$	Male	No	2	1.29E+02	1.485%	3.69E+01	5.94E+01	8.74E+01	1.39E+02	3.94E+02
	Vesselinovitch *et al.* (1979b)	Mouse*	$B6C3F_1$	Male	No	2	3.56E+02	8.154%	3.51E+01	6.18E+01	1.02E+02	2.14E+02	Indeterminate
Tetrachlorodibenzodioxin (TCDD)	Della Porta *et al.* (1987)	Mouse*	$B6C3F_1$	Female	Yes	2	1.88E+00	0.000%	1.36E-01	6.94E-01	1.46E+00	2.58E+00	5.19E+00
				Male	Yes	2	2.41E-01	0.000%	6.44E-02	1.53E-01	2.26E-01	3.11E-01	4.65E-01
Urethane	Choudari Kommineni *et al.* (1970)	Rat*	MRC	Male/ Female	Yes	2	1.39E+01	1.031%	4.95E+00	7.40E+00	1.02E+01	1.51E+01	3.56E+01
Vinyl chloride	Maltoni *et al.* (1981)	Rat	Sprague Dawley	Male/ Female	Yes	2	6.18E+00	0.000%	4.58E+00	5.41E+00	6.08E+00	6.85E+00	8.13E+00

* Later life exposure group was dosed during the later part of the juvenile period.
[a] Animals in the postnatal exposure group were dosed on day 1 of life.
[b] Animals in the postnatal exposure group were dosed on day 15 of life.
[c] Number of model parameters differed by tumor site.

Table B3. Multi-Lifestage Exposure Studies: Juvenile Lifestage Potency (LP) Ratios for Different Chemicals

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Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
7,12-Dimethyl-benz[a]anthracene (DMBA)	Meranze *et al.* (1969)	Rat	Fels-Wistar	Female	Yes	2	9.74E+00	0.248%	2.79E+00	4.37E+00	6.17E+00	9.24E+00	2.07E+01
				Male	No	2	1.24E+00	0.000%	3.21E-01	6.31E-01	9.95E-01	1.55E+00	2.96E+00
Dimethylnitrosamine (DMN)	Noronha and Goodall (1984)	Rat	CRL/CDF	Male	Yes	2	1.80E+00	0.000%	1.14E+00	1.46E+00	1.73E+00	2.07E+00	2.70E+00
3-Hydroxyxanthine	Anderson *et al.* (1978)	Rat	Wistar	Female	No	2	1.55E+00	1.551%	9.89E-02	4.81E-01	9.03E-01	1.63E+00	5.28E+00
Methylnitrosourea (MNU)	Grubbs *et al.* (1983)	Rat	Sprague Dawley	Female[a+]	Yes	2	3.57E+00	0.000%	2.25E+00	2.88E+00	3.43E+00	4.11E+00	5.39E+00
				Female[b]	Yes	2	1.11E+01	0.000%	6.61E+00	8.64E+00	1.05E+01	1.29E+01	1.77E+01
Urethane	Choudari Kommineni *et al.* (1970)	Rat*	MRC	Male/ Female	No	2	7.86E-01	1.031%	2.86E-02	2.92E-01	5.42E-01	9.41E-01	2.39E+00

* Later life exposure group was dosed during the later part of the juvenile period.

[+] MNU dataset selected for generation of juvenile LP ratio mixture distribution; see text for explanation.

[a] Animals in the adult exposure group were dosed from day 80 to 87.

[b] Animals in the adult exposure group were dosed from day 140 to 147.

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Appendix C

Sensitivity Analyses: Lifestage Potency (LP) Ratio and ASF Mixture Frequency Distributions for Multi-Lifestage Exposure Studies

This appendix presents the detailed findings for the LP ratio frequency distributions generated for the prenatal, postnatal, and juvenile lifestages from the multi-lifestage exposure studies. As described in the Methods section, in order to derive the LP ratio mixture distribution for each early lifestage, each chemical in the data set was equally likely to be sampled, and each chemical was represented by a single LP ratio distribution. When there were multiple LP ratios (representing multiple studies) on a chemical, the LP ratio distributions from all studies of that chemical were combined by equally sampling from each LP ratio distribution via Monte Carlo methods to obtain a single LP ratio distribution for that chemical. Sensitivity analyses were also conducted, employing alternative sampling methods to obtain a single LP ratio distribution to represent each chemical for which there were multiple studies. In one alternative sampling method, each of the LP ratio distributions available for a chemical is sampled based upon an inverse-variance weighting scheme, where the variance is calculated for the distribution of the logarithm of the LP ratio, Var[log LP ratio], and the likelihood that an LP ratio distribution is sampled is proportional to 1/Var(log[LP ratio]). In another alternative sampling method, the LP ratio distribution with the largest median is used as the representative "mixture" LP ratio distribution to represent the chemical.

Prenatal LP Ratio and ASF Mixture Distributions

Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

Figure C-1a shows the prenatal LP ratio mixture frequency distribution generated using this method. The frequency distribution is multi-modal (four modes), at 0.15, 0.54, 3.65, and 47.86. The largest peak of the frequency distribution is an LP ratio value of 0.54. The smallest mode, at an LP ratio value of 0.15, is primarily composed of LP ratio values from the following

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chemicals: di-*n*-propylnitrosamine, 2-hydroxypropylnitrosamine, and NNK. These chemicals display confidence intervals that indicate the true value of the LP ratio is statistically significantly less than 1.0 (at the 0.05 level; see also Fig. 6 in the main text). The second mode, with a value of 0.54, is comprised primarily of LP ratio values from chemicals whereby a bulk of their LP ratio distributions lie below 1.0, yet the 90% upper confidence bound may be slightly greater than 1.0. These chemicals are as follows: benzidine (female mouse), butylnitrosourea, DES, DEN (one of the two female hamster studies), dimethylnitrosamine, and 3-MC (one of the two female mouse studies). The third mode, with a value of 3.65, consists primarily of LP ratio values from chemicals whereby a bulk of their LP ratio distributions lie above 1.0 yet their upper 90% confidence bound is generally not greater than 10. These chemicals are as follows: DEN (one of the two female hamster studies), ENU (one of two female rat studies), 3-MC (one of the two female mouse studies), safrole (female mouse), urethane, and vinyl chloride. The largest mode is primarily composed of LP ratio values from the following chemicals: benzidine (male mouse), 1-ethylnitrosobiuret, ENU (male rat, one of two female rat studies), and safrole (male mouse). These chemicals display confidence intervals that indicate the true value of the LP ratio is statistically significantly greater than 1.0 (at the $p \leq 0.05$ level).

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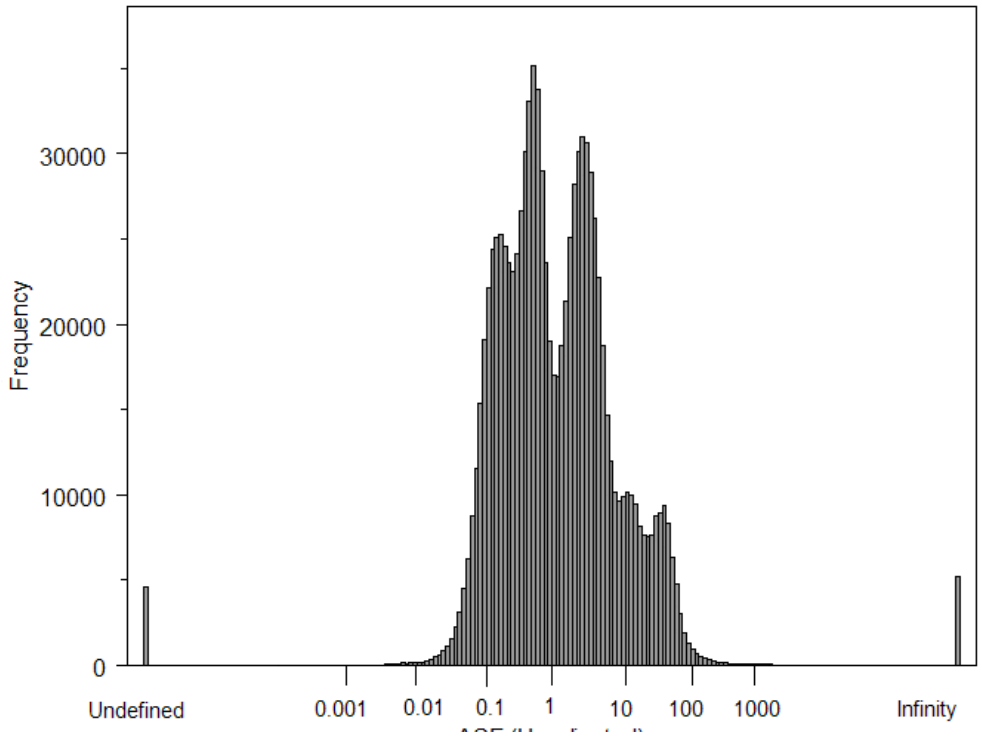
Figure C-1a. Prenatal LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies (Method presented in the main text)



Alternative Weighting: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies.

Figure C-1b shows the prenatal LP ratio mixture frequency distribution generated using the alternative weighting method whereby each of the LP ratio distributions available for a chemical is sampled based upon an inverse-variance weighting scheme (i.e., the likelihood that an LP ratio distribution is sampled is proportional to 1/Var(log[LP ratio]) (Method 2). The prenatal LP ratio mixture frequency distribution is multi-modal (four modes). The modes of the frequency distribution are 0.14, 0.52, 3.63, and 47.86. The largest peak of the frequency distribution is an LP ratio value of 0.52. The general shape of this prenatal LP ratio mixture frequency distribution is similar to that generated when multiple LP ratio distributions for a chemical are equally weighted. Of those chemicals that had more than a single LP ratio dataset representing them, unless there were appreciable fold-differences across the studies, datasets within a chemical were

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generally sampled from equally, even using this inverse-variance weighting method. In instances where there were fold differences across datasets within a chemical, this inverse-variance weighting method assigns datasets with the greatest variability (log space) the smallest weights in comparison to other datasets with less variability (log space). Chemicals that have multiple prenatal studies representing them that have fold-differences such that they are not equally sampled are benzidine, ENU, and safrole. The greatest departure between the LP ratio mixture frequency distributions generated using equal weighting within a chemical and inverse-variance weighting within a chemical is attributed to the datasets associated with these chemicals.

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Figure C-1b. Prenatal LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies (Method 2)

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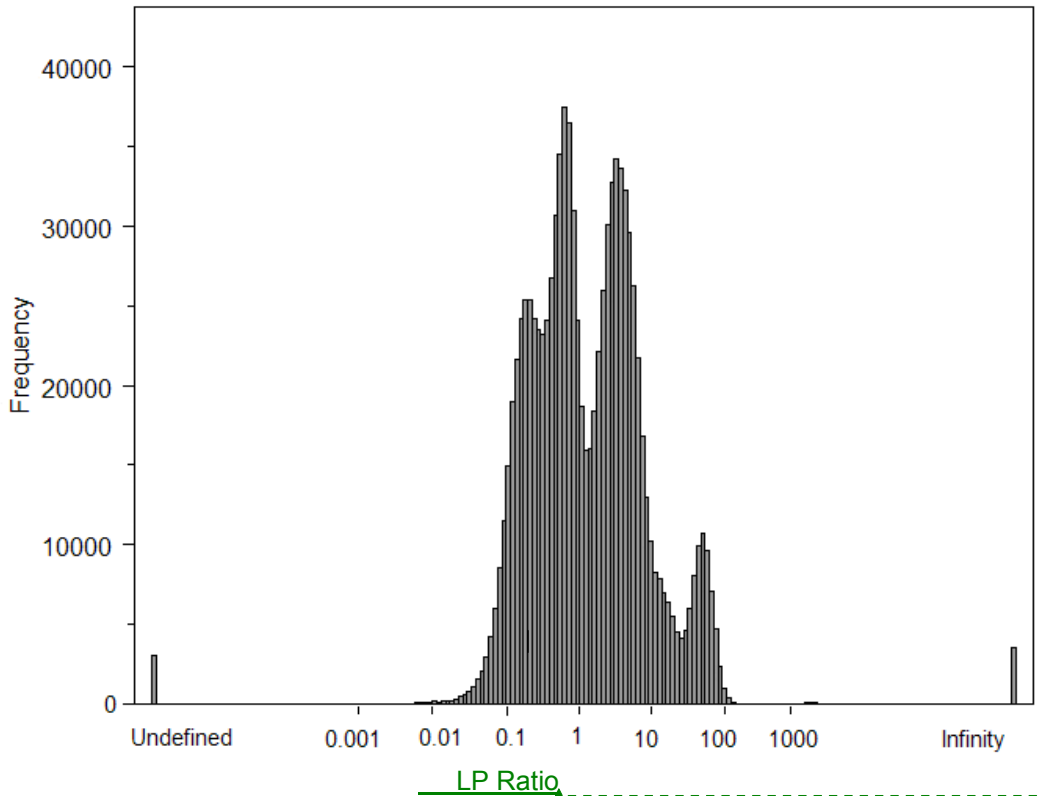

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Alternative Weighting: Chemicals Equally Weighted, Single Study Represents Each Chemical.

Figure C-1c shows the prenatal LP ratio mixture frequency distribution generated using the alternative weighting method whereby for chemicals with multiple studies LP ratios distributions, the distribution with the largest median was selected to represent that chemical in the LP ratio mixture distribution (Method 3). The prenatal LP ratio mixture frequency distribution is multi-modal (five modes). This distribution looks somewhat different than those shown in Figures C-1a and C-1b; it is more disperse and the modes of the distribution are more peaked for larger LP ratio values. The modes of this prenatal LP ratio mixture frequency distribution are 0.15, 0.53, 3.60, 19.12 and 47.98. The largest peak of this distribution is the LP ratio value of 0.53.

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Of those chemicals that had more than a single study representing them, the study with the largest median tended to also have the largest variance. As a result, the mixture frequency distribution resulting from Method 3 tends to be more spread out and shifted toward the right. The chemicals contributing to the peak with value 0.15 are di-n-propylnitrosamine, 2-hydroxypropylnitrosamine, and NNK. The chemicals primarily contributing to the mode with value 0.53 are butylnitrosourea and DES. The next largest peak with a value of 3.60 is comprised of the chemicals DEN, dimethylnitrosamine, 3-MC, urethane and vinyl chloride. The peaks with the largest modes (values of 19.12 and 47.98) consist of the chemicals benzidine, 1-ethylnitrosobiuret, ENU, and safrole. All of the studies that comprise the two peaks with the largest modes display confidence intervals that indicate the true value of the LP ratio is statistically significantly greater than 1 (at the 0.05 level).

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Figure C-1c. Prenatal LP Ratio Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical(Method 3)

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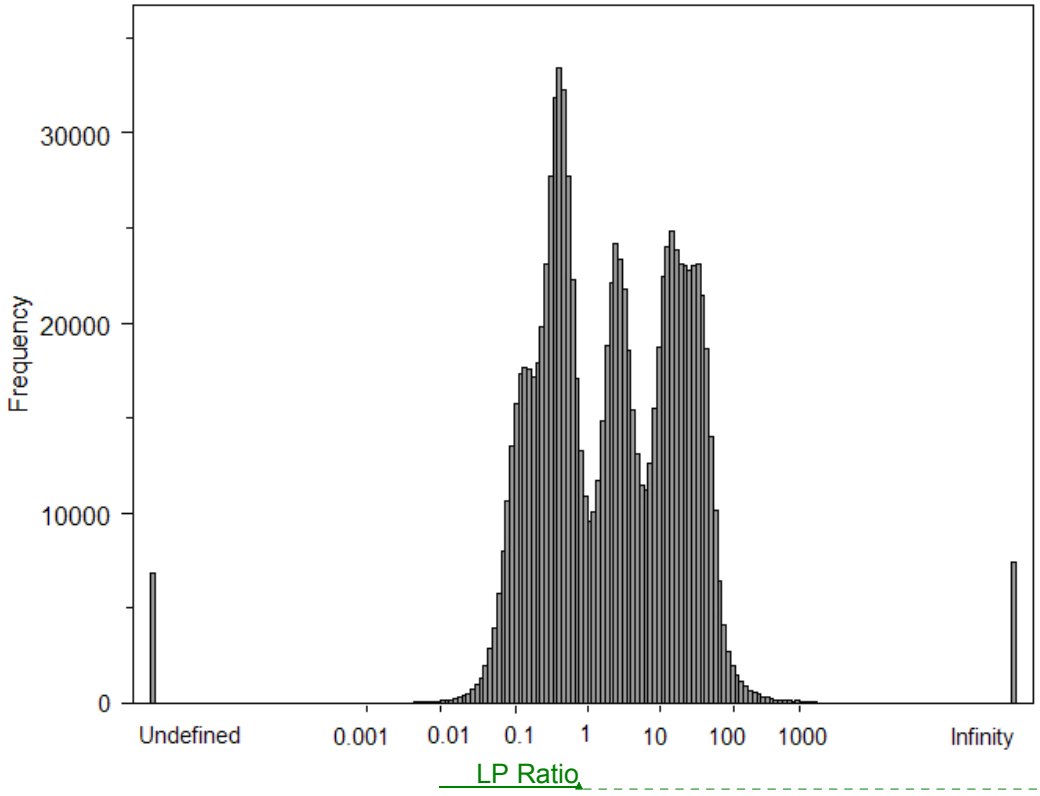

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The mean, and specific percentiles for the LP ratio and ASF mixture distributions for each method are provided in Table C-1. For the 30th percentile and below there is essentially no difference between the LP ratio mixture distributions across the methods. Slight differences between Method 1 and Method 2 appear at the latter percentiles, at the 80th percentile and greater. For percentiles greater than the 30th, the prenatal LP ratio mixture distribution derived via Method 3 has percentile values that are larger than the other methods. The distribution derived via Method 1 falls between Methods 2 and 3. These prenatal LP ratio mixture cumulative distribution functions follow a predictable pattern that is explained via the mixing algorithms employed. In summary, the LP ratio and ASF distributions generated by each of the three methods are multimodal with modes above and below unity.

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Table C-1. Prenatal LP Ratio and ASF Mixture Distribution Statistics by Method

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Statistics	LP Ratio			ASF		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Mean*	7.03	5.54	13.73	21.09	16.62	37.07
Percentiles						
5th	0.09	0.09	0.10	0.27	0.27	0.30
10th	0.12	0.13	0.15	0.36	0.39	0.45
20th	0.22	0.22	0.32	0.66	0.66	0.96
30th	0.38	0.39	0.50	1.14	1.17	1.50
40th	0.58	0.58	0.89	1.74	1.74	2.67
50th	0.96	0.93	2.49	2.88	2.79	7.47
60th	1.95	1.92	4.68	5.85	5.76	14.04
70th	3.11	2.96	12.39	9.33	8.88	37.17
80th	5.18	4.57	22.11	15.54	13.71	66.33
90th	16.52	11.18	40.35	49.56	33.54	121.05
95th	38.49	36.15	57.28	115.47	108.45	171.84

* Calculated excluding large values above the 99th percentile.

Postnatal LP Ratio and ASF Mixture Distributions

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Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

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Figure C-2a shows the postnatal LP ratio mixture frequency distribution generated using this method. The LP ratio frequency distribution has three modes, at 0.61, 8.66, and 96.49, with the largest peak at 8.66. The smallest mode, with a value of 0.61, is primarily composed of LP ratio values from the two studies with the 95% upper bound below the LP ratio value of 1.0. The second mode, with a value of 8.66, is comprised primarily of LP ratio values from chemicals with the bulk of their LP ratio distributions above one, but 95% upper confidence bounds less than 10: benzo[a]pyrene, butylnitrosourea, DEN, ENU, 3-MC, and MNU. The LP ratios for studies on these chemicals contribute the majority of the mass at the center of the distribution. The third mode, with a value of 96.49, consists primarily of chemicals with LP ratio values centered around 100: benzidine (one male mouse study), dibutylnitrosamine, and safrole. The LP ratios for these cases are statistically significantly greater than 10 (at the $p = 0.05$ level).

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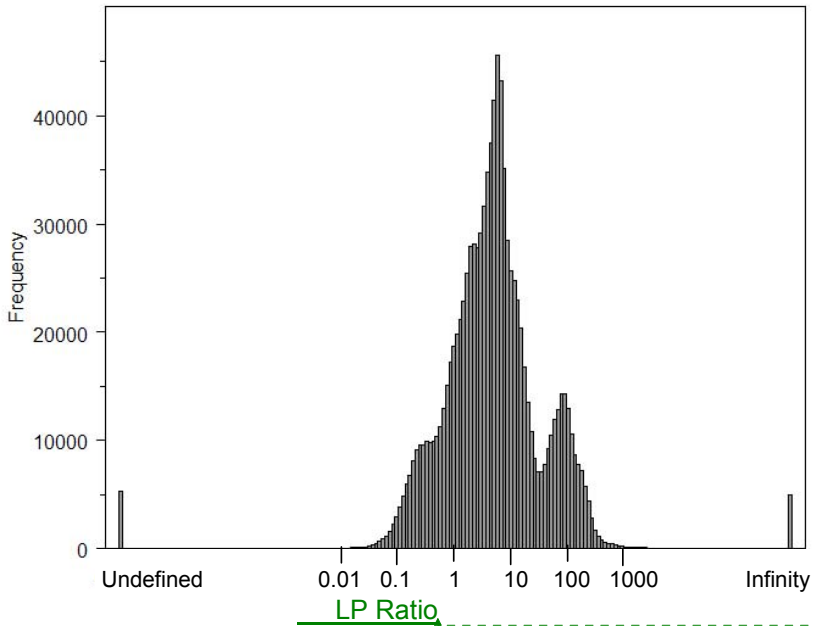
Figure C-2a. Postnatal LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies (Method 1, presented in the main text)



Alternative Weighting: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies.

Figure C-2b shows the postnatal LP ratio mixture frequency distribution generated using the alternative weighting method whereby each of the LP ratio distributions available for a chemical is sampled based upon an inverse-variance weighting scheme (i.e., the likelihood that an LP ratio distribution is sampled is proportional to 1/var(log[lp ratio]) (Method 2). The postnatal ASF mixture frequency distribution has four modes, at 0.49, 1.43, 8.66, and 95.55. As with Method 1, the largest has an LP ratio value of 8.66, and its general shape is similar to the one generated using Method 1 (Figure C-2a). The main difference is that the Method 2 distribution is slightly more spread out with more defined peaks, and the peaks tend to be more elevated. The higher peaks are due to the studies within a chemical that have smaller fold differences being weighted more heavily than those studies with greater variability (e.g. benzidene, benzo[a]pyrene, DEN, and ENU). However, the studies with greater variability (log space) are still contributing to the frequency distribution. The studies with the most variability (log space) and the largest LP ratio values contribute to the enhanced variability of Method 2 as compared to Method 1.

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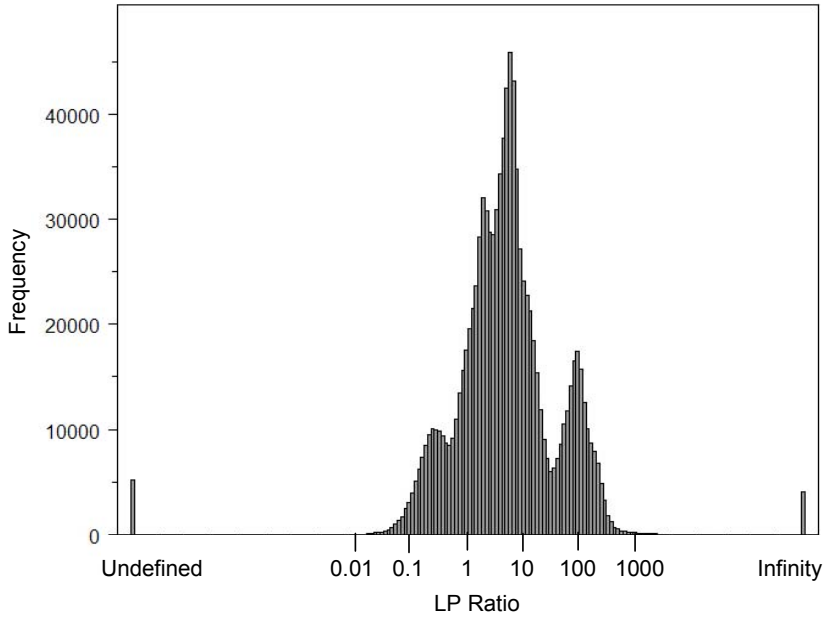
Figure C-2b. Postnatal LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies (Method 2)



Alternative Weighting: Chemicals Equally Weighted, Single Study Represents Each Chemical. Figure C-2c shows the postnatal LP ratio mixture frequency distribution generated using the alternative weighting method whereby for chemicals with multiple studies LP ratios distributions, the distribution with the largest median was selected to represent that chemical in the LP ratio mixture distribution (Method 3). The postnatal LP ratio mixture frequency distribution again has four modes, 0.58, 8.96, 97.83, and 163.79. It has two very distinct peaks and is more skewed to the right than those shown in Figures C-2a and C-2b. The largest peak of this frequency distribution is an LP ratio value of 8.96.

For chemicals where there is significant study-to-study variability, the effect of selecting the distribution with the largest median exaggerates the percentiles of the resultant mixture frequency distribution. This effect is most pronounced for the chemicals benzidine, DEN, DMBA, ENU, and β-propiolactone.

Figure C-2c. Postnatal LP Ratio Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical (Method 3)

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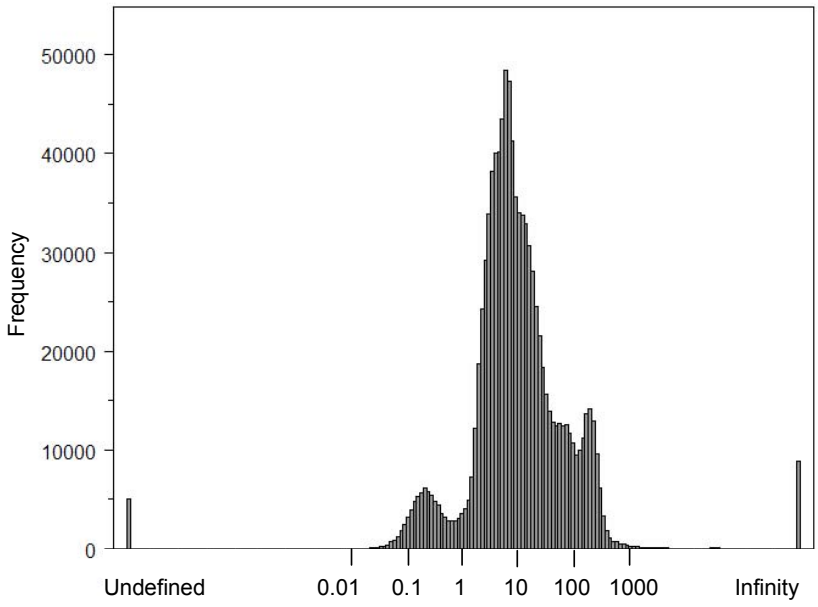

The mean, and specific percentiles for the LP ratio and ASF mixture distributions for each method are provided in Table C-2. The LP ratio and ASF distributions for Method 1 and Method 2 are nearly identical up to the 70th percentile. After the 70th percentile, Method 2 has slightly larger values as compared to Method 1. The most compact postnatal LP ratio distributions generally have values that are significantly greater than unity. As a result, the inverse-variance method (Method 2) produces a LP ratio mixture distribution that is shifted slightly to the right of the distribution derived using Method 1, where equal weighting is given to all studies within a chemical (see Figure 11 in the main text). The magnitude of this rightward shift with Method 2 is not particularly large however because there were no single studies amongst those chemicals with multiple studies with considerably smaller variances than the others in the set. The postnatal LP ratio and ASF mixture cumulative distributions derived via Method 3 have percentile values that are considerably larger than the other methods beyond the 5th percentile. The most peaked mode of the postnatal LP ratio mixture frequency distribution is similar across the mixing algorithms employed (i.e., Methods 1-3). However, when a single study with the largest median value is selected to represent the chemical (Method 3), the percentiles of the distribution become somewhat larger as compared to that seen using Methods 1 or 2.

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Table C-2. Postnatal LP Ratio and ASF Mixture Distribution Statistics by Method

Statistics	LP Ratio			ASF		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Mean*	27.08	27.62	42.45	78.53	80.10	123.11
Percentiles						
5th	0.20	0.20	0.26	0.58	0.58	0.75
10th	0.41	0.40	1.48	1.19	1.16	4.29
20th	1.08	1.14	2.80	3.13	3.31	8.12
30th	1.93	1.94	4.01	5.60	5.63	11.63
40th	3.13	3.10	5.54	9.08	8.99	16.07
50th	4.64	4.61	7.45	13.46	13.37	21.61
60th	6.35	6.29	11.00	18.42	18.24	31.90
70th	9.62	9.60	16.99	27.90	27.84	49.27
80th	18.10	19.71	33.58	52.49	57.16	97.38
90th	72.78	81.79	106.08	211.06	237.19	307.63
95th	122.82	129.22	188.14	356.18	374.74	545.61

* Calculated excluding large values above the 99th percentile.

Juvenile LP Ratio and ASF Mixture Distributions

Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

Figure C-3a shows the juvenile LP ratio mixture frequency distribution generated using this method. The frequency distribution is bi-modal, with modes at 1.58 and 2.05. The largest peak of the distribution is an LP ratio value of 1.58. By sorting the chemicals from smallest to largest based upon the value of the lower confidence bound, we can approximately determine each chemical's contribution to the percentiles of the LP ratio mixture frequency distribution. Urethane and 3-hydroxyxanthine are the largest contributors to the lower percentiles of the mixture frequency distribution. Conversely, MNU and the DMBA female rat datasets are the largest contributors to the highest percentiles of the mixture frequency distribution. The male rat DMBA dataset and the DMN dataset (also in male rats) comprise the middle area of the distribution.

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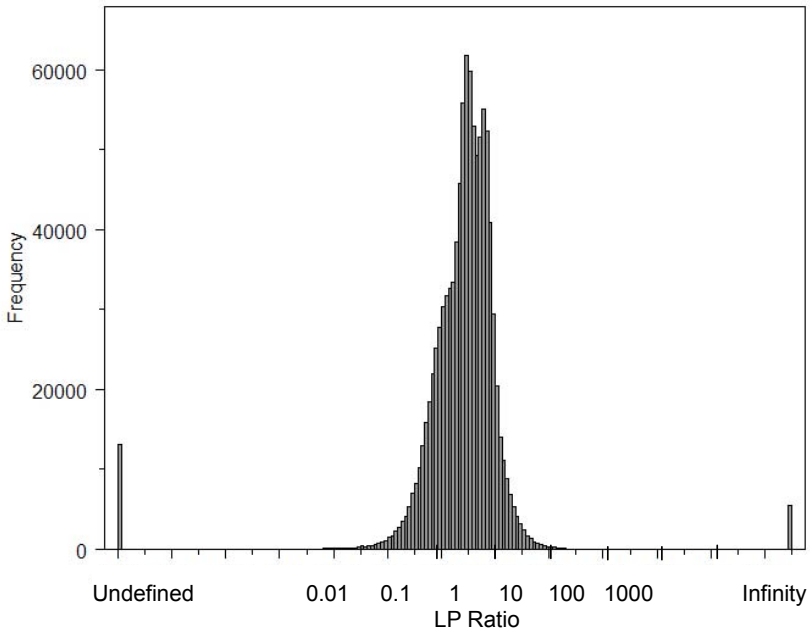
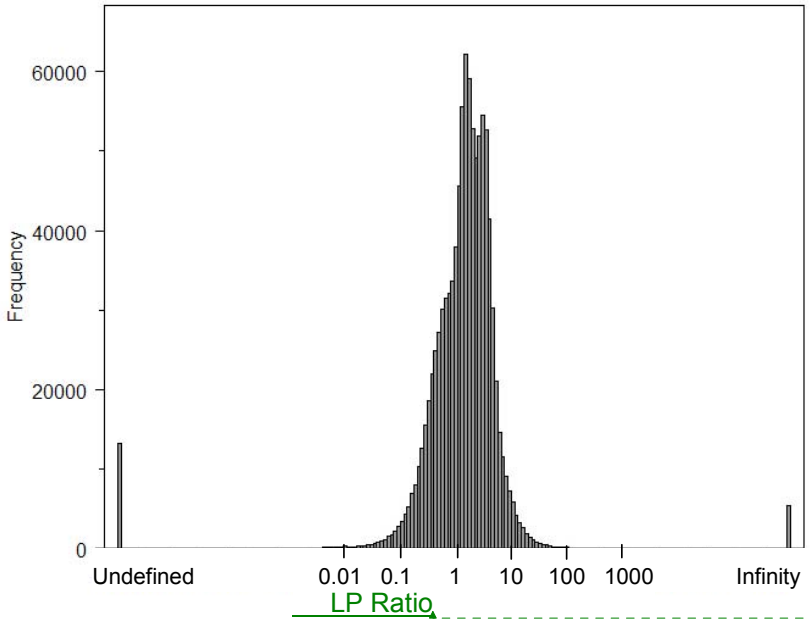
Figure C-3a. Juvenile LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies (Method 1)



Alternative Weighting: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies.

Figure C-3b shows the juvenile LP ratio mixture frequency distribution generated using the alternative weighting method whereby each of the LP ratio distributions available for a chemical is sampled based upon an inverse-variance weighting scheme (i.e., the likelihood that an LP ratio distribution is sampled is proportional to 1/var(log[lp ratio]) (Method 2). The frequency distribution is bi-modal, with modes at 1.57 and 2.08. The largest peak of the distribution is an LP ratio value of 1.57. This LP ratio distribution is practically identical to the LP ratio distribution derived via Method 1.

Figure C-3b. Juvenile LP Ratio Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies (Method 2)



Alternative Weighting: Chemicals Equally Weighted, Single Study Represents Each Chemical.

Figure C-3c shows the juvenile LP ratio mixture frequency distribution generated using the alternative weighting method whereby for chemicals with multiple studies LP ratios distributions, the distribution with the largest median was selected to represent that chemical in the LP ratio mixture distribution (Method 3). The juvenile LP ratio mixture frequency distribution is bi-modal, and looks similar to that generated by Methods 1 and 2. However, the modes of this distribution, 1.59 and 2.37, are less peaked and are of similar height. The largest peak of this mixture frequency distribution is the LP ratio value of 2.37.

Figure C-3c. Juvenile LP Ratio Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical (Method 3)

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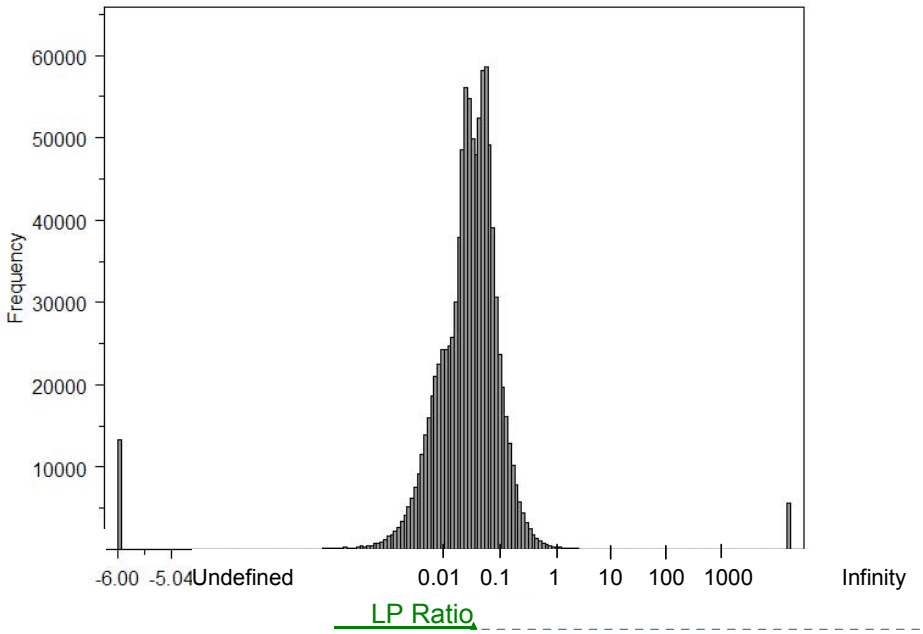

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The mean, and certain percentiles for each method are provided in Table C-3. The juvenile LP ratio and ASF mixture cumulative distributions derived via Method 1 are nearly indistinguishable from the mixture cumulative distributions derived via Method 2. The comparative length of the boxplots and their associated 90% confidence intervals between the DMBA exposed male and female rat bioassay studies (shown in Figure 14 of the main text) are similar such that the inverse-variance weighting method produces a nearly identical LP ratio ad ASF mixture distributions in comparison to Method 1. Method 3 results in greater differences in the LP ratio and ASF mixture distributions as compared to Methods 1 and 2 because the female DMBA rat LP ratio (and ASF) distribution is solely being sampled to represent the chemical DMBA. The female DMBA rat LP ratio distribution consists of LP ratio values that are entirely above unity. The difference observed is reflective of the greater sensitivity of female rats to mammary (i.e., breast) cancer during the juvenile period.

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Table C-3. Juvenile LP Ratio and ASF Mixture Distribution Statistics by Method

Statistics	LP Ratio			ASF		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Mean*	2.63	2.71	3.49	7.10	7.32	9.42
Percentiles						
5th	0.20	0.20	0.20	0.54	0.54	0.54
10th	0.34	0.34	0.36	0.92	0.92	0.97
20th	0.60	0.61	0.69	1.62	1.65	1.86
30th	0.93	0.94	1.16	2.51	2.54	3.13
40th	1.31	1.33	1.58	3.54	3.59	4.27
50th	1.67	1.68	2.03	4.51	4.54	5.48
60th	2.10	2.13	2.69	5.67	5.75	7.26
70th	2.77	2.80	3.44	7.48	7.56	9.29
80th	3.57	3.62	4.43	9.64	9.77	11.96
90th	4.96	5.04	6.74	13.39	13.61	18.20
95th	7.29	7.46	10.16	19.68	20.14	27.43

* Calculated excluding large values above the 99th percentile.

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Appendix D

DEN Case Study: Cancer Potency Distributions for DEN Single—Lifestage Exposure Experiments and Sensitivity Analyses

DEN (diethyl-N-nitrosoamine) cancer potency distribution statistics derived from cancer bioassay single-lifestage exposure experiments conducted in mice exposed to DEN during either the prenatal, postnatal, or juvenile lifestage are presented here. Table D1 presents the cancer potency distributions and study details for the prenatal exposure datasets. Table D2 presents the cancer potency distributions and study details for the postnatal exposure datasets. Table D3 presents the cancer potency distributions and study details for the juvenile exposure datasets.

The remainder of this appendix presents the detailed findings for the LP_i ratio and ASF_i cumulative distribution functions generated for the prenatal and postnatal lifestages from the DEN single-lifestage exposure experiments in mice. As described in the Methods section, an overall distribution of the logarithm of potencies was created for each lifestage. This was accomplished via Monte Carlo methods, by sampling from each of the individual (log) potency distributions derived for each experiment for that exposure period equally. Sensitivity analyses were also conducted, employing alternative sampling methods to create the potency distribution for a given lifestage. One alternative method truncated each individual potency distribution at the fifth and ninety-fifth percentiles prior to creating the equally weighted potency mixture distribution. A second alternative method sampled from the potency distributions based upon weights equal to the computed inverse-variance of each (logarithm) potency distribution. That is, the variance was calculated for the distribution of the logarithm of the q_1, $Var[\log q_1]$. The likelihood that an q_1 is sampled is proportional to $1/Var(\log[q_1])$. A third alternative method sampled from the potency distributions based upon weights equal to the computed interquartiles (25^{th} and 75^{th} percentiles) of each (logarithm) potency distribution. The likelihood that an q_1 is sampled is proportional to $1/\log(q_{1,75}) - \log(q_{1,25})$. Potency mixture distributions for each lifestage were obtained using each of these methods. The LP_i ratio and ASF_i distributions computed using potency mixture distributions derived via the various sampling methods are presented.

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Table D1. DEN Prenatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Anderson *et al.* (1989)	C3H/HeN	Female (540)[a]	0.0793739	0.0147744	0.0558226	0.0690785	0.0788648	0.0891974	0.10467
		Female (650)[a]	0.00135364	0.00149944	0	0.000151185	0.00091793	0.00200131	0.00440449
		Male (461)[a]	0.138321	0.0511987	0.0596149	0.101144	0.134968	0.171184	0.229449
		Male (644)[a]	0.00411408	0.00501051	0	0.000575143	0.00236785	0.0054626	0.0151794
Mohr and Althoff (1965)	NMRI	Female	0.239892	0.067558	0.132634	0.192885	0.236059	0.283579	0.359286
		Male	0.187186	0.0701371	0.0756144	0.137731	0.18516	0.233584	0.306676
Vesselinovitch (1983)	$B6C3F_1$	Female	0.00667806	0.00582567	0	0.00240222	0.00513474	0.00941687	0.0187249
		Male	0.00952546	0.00812867	0	0.00313266	0.00792549	0.0140185	0.0251028

[a] Day of sacrifice.

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Table D2. DEN Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Boberg *et al.* (1983)	$B6C3F_1$	Male	48.3648	14.391	28.9842	38.0081	46.0943	56.3092	75.2761
Drinkwater and Ginsler (1986)	$B6C3F_1$	Male	17.9418	4.73622	11.1826	14.5018	17.3425	20.7377	26.8706
	C3H/HeJ	Male	22.9791	7.17883	13.3745	17.8596	21.7639	26.8271	36.8345
			2.68143	0.555517	1.82759	2.28691	2.63839	3.03852	3.65828
Lai *et al.* (1985)	$B6C3F_2$	Male	12.8913	2.39873	9.27649	11.1601	12.6851	14.41	17.2813
Rao and Vesselinovitch (1973)	$B6C3F_1$	Female	1.41599	0.285257	0.978519	1.21049	1.39454	1.59902	1.93235
		Male	2.30206	0.661882	1.43057	1.82508	2.18762	2.65531	3.542
Turusov *et al.* (1973)	CF-1	Female	0.575921	0.131105	0.37814	0.481996	0.565362	0.65859	0.810561
		Male	0.830932	0.174098	0.565934	0.707333	0.818634	0.941348	1.13883
Vesselinovitch *et al.* (1984)	$B6C3F_1$	Female (Day 1)[a]	0.589447	0.062358	0.491627	0.545742	0.586343	0.63034	0.697702
		Male (Day 1)[a]	1.03983	0.148035	0.808426	0.93473	1.03146	1.13696	1.29788
		Female (Day 15)[b]	0.453917	0.051127	0.374289	0.417738	0.451081	0.48722	0.543127
		Male (Day 15)[b]	0.894762	0.115637	0.717843	0.81268	0.887008	0.968949	1.09932

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Table D2. Continued. DEN Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Vesselinovitch *et al.* (1984)	$C3AF_1$	Female (Day 1) [a]	0.641045	0.111376	0.469409	0.562094	0.634021	0.712722	0.837305
		Male (Day 1) [a]	1.11429	0.173993	0.835839	0.993194	1.10931	1.22972	1.41043
		Female (Day 15) [b]	0.303322	0.050107	0.224424	0.267995	0.300956	0.336305	0.390135
		Male (Day 15) [b]	0.649307	0.106642	0.480839	0.574691	0.644526	0.719069	0.834195
Vesselinovitch (1980)	$B6C3F_1$	Male	3.07401	0.452323	2.36812	2.75378	3.04908	3.3669	3.88832

[a] Mice were dosed on day 1 of life.
[b] Mice were dosed on day 15 of life.

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Table D3. DEN Juvenile Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Rao and Vesselinovitch (1973)	$B6C3F_1$	Male	0.093411	0.031799	0.048166	0.070136	0.089942	0.113182	0.15094
Vesselinovitch *et al.* (1984)	$B6C3F_1$	Female	0.267868	0.049742	0.191397	0.232495	0.26428	0.299789	0.356424
		Male	0.203009	0.029221	0.157292	0.182313	0.201531	0.22207	0.254173
	$C3AF_1$	Female	0.555707	0.178219	0.313719	0.427389	0.527989	0.654242	0.88766
		Male	0.40558	0.094585	0.268191	0.337805	0.395187	0.462985	0.579307

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DEN Prenatal and Postnatal LP_i Ratio and ASF_i Mixture Distributions

Equal Weighting of Potency Distributions, without or with Truncation.

In the variation on Method 1, where the potency distributions derived from each experiment are truncated at the 5th and 95th percentiles, the results for the LP_j ratio distributions are not appreciably different from those obtained without the truncation, and indicate the same general conclusions (Table D4).

Table D4. DEN Prenatal and Postnatal LP_j Ratio Distributions – Equal Weighting of Potency Distributions
Method 1, as presented in the main text, and Method 1 (truncated)

Percentiles	Method 1		Method 1 (truncated)	
	Prenatal LP_j Ratio	Postnatal LP_j Ratio	Prenatal LP_j Ratio	Postnatal LP_jRatio
5th	0.00	0.74	0.00	0.76
10th	0.002	0.96	0.002	0.98
20th	0.008	1.50	0.007	1.51
30th	0.02	2.19	0.01	2.20
40th	0.03	3.00	0.03	2.98
50th	0.10	4.21	0.10	4.21
60th	0.35	6.01	0.36	5.99
70th	0.53	9.53	0.53	9.31
80th	0.75	47.51	0.74	46.84
90th	1.08	240.62	1.06	239.10
95th	1.36	408.95	1.30	393.52

Figure D-1 shows the DEN prenatal and postnatal LP_j ratio frequency distributions generated using Method 1. Both the prenatal and postnatal LP_j ratio frequency distributions are multi-modal.

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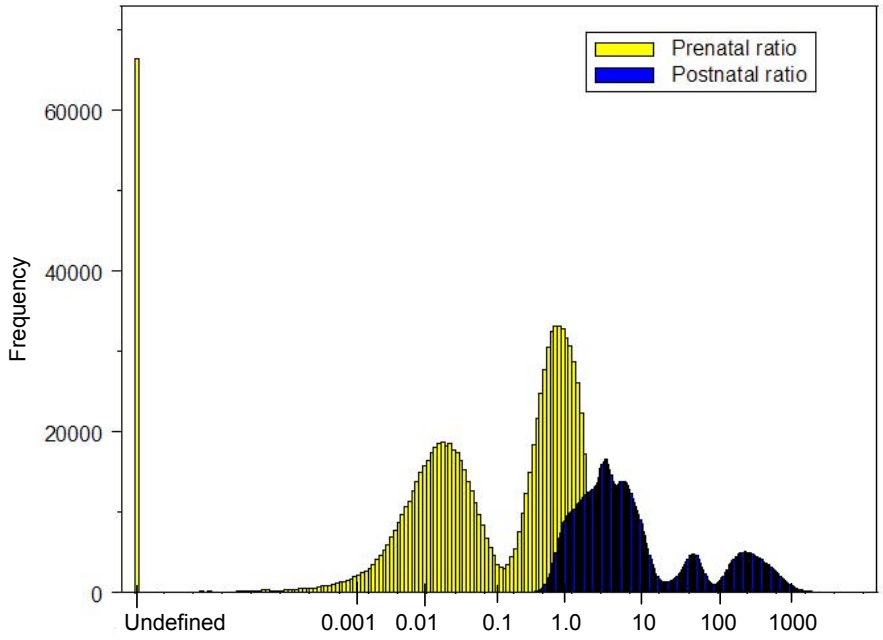
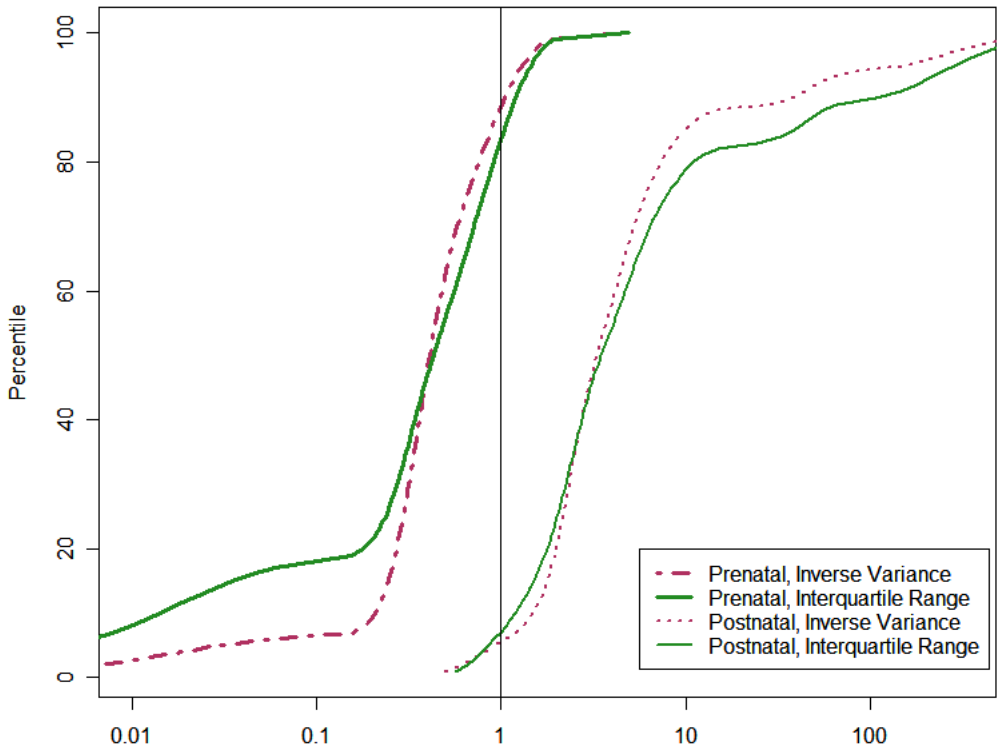
Figure D-1. Method 1 DEN Prenatal and Postnatal LP_i Ratio Frequency Distributions – Equal Weighting of Potency Distributions



Alternative Weighting: Weighting Potency Distributions by Inverse-Variance and the Interquartile Range.

Figure D-2 shows the DEN prenatal and postnatal LP_i ratio cumulative distribution functions generated using Method 2a, weighting by inverse-variance, and Method 2b, weighting by the interquartile range (IQR). Qualitatively the results are similar to Method 1, with considerable sensitivity exhibited in the postnatal lifestage. The magnitude of the differences in the LP_i ratio distributions for DEN in the prenatal and postnatal lifstages is evident.

Figure D-2. Methods 2a and 2b DEN Prenatal and Postnatal LP_i Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions



The percentiles for the DEN prenatal and postnatal LP_i ratio distributions are provided in Table D5a, and the percentiles for the DEN prenatal and postnatal ASF_i distributions are provided in Table D5b. With inverse-variance weighting, slightly less than 89% of the prenatal LP_i ratio distribution lies below the value of one. Although not statistically significant, the distributional statistics suggest that mice exposed during the prenatal lifestage are less prone to the tumorigenic effects of DEN as compared to those exposed as juveniles. For the postnatal LP_i ratio distribution, more than 94% of the distribution is greater than unity under Method 2a (inverse-variance weighting), indicating that mice exposed during the postnatal lifestage are more prone to the tumorigenic effects of DEN than those exposed as juveniles. The distributional

differences in cancer risk (as compared to juveniles) between DEN exposures occurring during the prenatal lifestage versus the postnatal lifestage are quite evident.

Table D5a. Method 2 DEN Prenatal and Postnatal LP_i Ratio Distributions – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal LP_i Ratio	Postnatal LP_i Ratio	Prenatal LP_i Ratio	Postnatal LP_i Ratio
5th	0.03	0.93	0.005	0.85
10th	0.21	1.49	0.01	1.19
20th	0.27	1.99	0.18	1.77
30th	0.32	2.34	0.27	2.24
40th	0.36	2.74	0.34	2.73
50th	0.41	3.31	0.43	3.48
60th	0.47	4.18	0.55	4.71
70th	0.58	5.27	0.71	6.42
80th	0.75	7.36	0.91	11.02
90th	1.04	37.80	1.20	106.70
95th	1.30	154.34	1.45	287.68

Table D5b. Method 2 DEN Prenatal and Postnatal ASF_i Distributions – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal ASF_i	Postnatal ASF_i	Prenatal ASF_i	Postnatal ASF_i
5th	0.09	2.69	0.02	2.47
10th	0.62	4.33	0.03	3.45
20th	0.81	5.78	0.54	5.13
30th	0.95	6.80	0.81	6.50
40th	1.08	7.94	1.02	7.92
50th	1.23	9.60	1.29	10.09
60th	1.42	12.12	1.65	13.66
70th	1.73	15.27	2.13	18.62
80th	2.25	21.35	2.73	31.96
90th	3.13	109.62	3.60	309.43
95th	3.90	447.59	4.35	834.27

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Appendix E

ENU Case Study: Cancer Potency Distributions for ENU Single-Lifestage Exposure Experiments, and Sensitivity Analyses

ENU (N-ethyl-N-nitrosourea) cancer potency distribution statistics derived from cancer bioassay single-lifestage exposure experiments conducted in mice exposed to ENU during either the prenatal, postnatal, or juvenile lifestage are presented here. Table E1 presents the cancer potency distributions and study details for the prenatal exposure datasets. Table E2 presents the cancer potency distributions and study details for the postnatal exposure datasets. Table E3 presents the cancer potency distributions and study details for the juvenile exposure datasets.

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The remainder of this appendix presents the detailed findings for the LP_j ratio and ASF_j cumulative distribution functions generated for the prenatal and postnatal lifestages from the ENU single-lifestage exposure experiments in mice. As described in the Methods section, an overall distribution of the logarithm of potencies was created for each lifestage. This was accomplished via Monte Carlo methods, by sampling from each of the individual (log) potency distributions derived for each experiment for that exposure period equally. Sensitivity analyses were also conducted, employing alternative sampling methods to create the potency distribution for a given lifestage. One alternative method truncated each individual potency distribution at the fifth and ninety-fifth percentiles prior to creating the equally weighted potency mixture distribution. A second alternative method sampled from the potency distributions based upon weights equal to the computed inverse-variance of each (logarithm) potency distribution. That is, the variance was calculated for the distribution of the logarithm of the q_1, $Var[\log q_1]$. The likelihood that an q_1 is sampled is proportional to $1/Var(\log[q_1])$. A third alternative method sampled from the potency distributions based upon weights equal to the computed interquartiles (25^{th} and 75^{th} percentiles) of each (logarithm) potency distribution. The likelihood that an q_1 is sampled is proportional to $1/\log(q_{1\_75}) - \log(q_{1\_25})$. Potency mixture distributions for each lifestage were obtained using each of these methods. The LP_j ratio and ASF_j distributions computed using potency mixture distributions derived via the various sampling methods are presented.

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Table E1. ENU Prenatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Study	Strain	Gender	Mean	SD	Percentiles 5th	25th	50th	75th	95th
Diwan *et al.* (1974)	AKR/J x SWR/J	Female	1.52277	0.531149	0.796624	1.14448	1.44501	1.81488	2.52135
		Male	0.788833	0.242592	0.408852	0.617535	0.777224	0.946775	1.21021
	SWR/J x AKR/J	Female	6.40048	1.26518	4.48233	5.50555	6.30745	7.19692	8.64127
		Male	8.23676	2.02853	5.41389	6.7886	7.96423	9.37737	12.0313
Kauffman (1976)	Swiss	Female (Day -7)[a]	2.75745	0.780679	1.70604	2.20068	2.63687	3.17936	4.26637
		Female (Day -6)[a]	2.73481	0.777314	1.68615	2.1712	2.60713	3.15855	4.24545
		Female (Day -5)[a]	2.39602	0.773729	1.38175	1.83596	2.26666	2.8147	3.8993
		Female (Day -4)[a]	2.79589	0.781762	1.74426	2.23081	2.66744	3.2193	4.3065
		Female (Day -3)[a]	2.53857	0.770214	1.50408	1.98352	2.41683	2.95218	3.99008
Vesselinovitch *et al.* (1977)	$B6C3F_1$	Female (Day -10)[a]	0.042928	0.00468297	0.0354941	0.0396959	0.0427621	0.0460137	0.0509013
		Female (Day -8)[a]	0.0886033	0.00963707	0.0736767	0.0817998	0.0880656	0.0948531	0.105386
		Female (Day -6)[a]	0.136846	0.0191498	0.107902	0.123231	0.135315	0.148804	0.171023
		Female (Day -4)[a]	0.083219	0.0122441	0.0645201	0.0744767	0.0823669	0.0910178	0.104993
		Male (Day -10)[a]	0.0508204	0.00566404	0.041823	0.0468659	0.0506208	0.0545794	0.0604567
		Male (Day -8)[a]	0.127622	0.0154249	0.103632	0.116711	0.126869	0.137618	0.154515
		Male (Day -6)[a]	0.286018	0.0598357	0.204919	0.243175	0.277137	0.319002	0.398503
		Male (Day -4)[a]	0.165365	0.0228038	0.131436	0.149108	0.16331	0.179562	0.206382
	$C3B6F_1$	Female (Day -10)[a]	0.0235324	0.00539875	0.0151785	0.0197164	0.0232224	0.0270231	0.0329388
		Female (Day -8)[a]	0.111417	0.0169991	0.0860396	0.0992914	0.109892	0.121913	0.141993
		Female (Day -6)[a]	0.121747	0.0240692	0.0860168	0.104546	0.119536	0.13667	0.165114
		Female (Day -4)[a]	0.0729087	0.00911406	0.0587817	0.0664352	0.0723775	0.078822	0.0889698
		Male (Day -10)[a]	0.0356864	0.00744729	0.0242335	0.0304194	0.0352127	0.0404608	0.0488031
		Male (Day -8)[a]	0.167691	0.0313038	0.122511	0.14528	0.164215	0.186164	0.225405
		Male (Day -6)[a]	0.241567	0.0548256	0.167721	0.202249	0.233152	0.271325	0.345658
		Male (Day -4)[a]	0.083293	0.00962997	0.068251	0.0765158	0.0828024	0.0895821	0.0999465
Vesselinovitch (1983)	$B6C3F_1$	Female	0.0188286	0.00433908	0.0123229	0.0156683	0.0184721	0.0215981	0.0266903
		Male	0.0311922	0.00595895	0.0220985	0.0269647	0.0307819	0.0350559	0.041544
Wiggenhauser and Schmahl (1987)	NMRI	Male & Female (Day -8)[a]	0.191795	0.0154062	0.166844	0.181108	0.191371	0.202129	0.217871
		Male & Female (Day -7)[a]	0.181807	0.0165413	0.155072	0.170363	0.181426	0.192894	0.209776
		Male & Female (Day -6)[a]	0.153851	0.0149143	0.129937	0.143407	0.153387	0.16381	0.179375

[a] Day of dosing in gestation, where day of birth is designated as day 1.

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Table E2. ENU Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Study	Strain	Gender	Mean	SD	Percentiles				
					5th	25th	50th	75th	95th
Anderson *et al.* (1989)	C3H/HeNCr MTV	Female (405)[a]	0.401602	0.0714822	0.295997	0.350593	0.394317	0.445627	0.531848
		Female (451)[a]	0.190949	0.0332242	0.139564	0.16738	0.18888	0.212447	0.249345
		Male (342)[a]	0.705296	0.160744	0.46968	0.589517	0.689687	0.803716	0.997279
		Male (397)[a]	0.409096	0.0684954	0.300275	0.361199	0.406761	0.454284	0.526448
Drinkwater and Ginsler (1986)	C3H/HeJ	Male	1.87256	0.619931	1.04439	1.42364	1.7664	2.21011	3.03312
	C57BL/6J	Male	0.193632	0.0706384	0.0924212	0.141821	0.185241	0.236855	0.326457
Naito *et al.* (1982)	A/He	Female	0.488979	0.113488	0.321961	0.407412	0.47754	0.558629	0.696155
		Male	0.53121	0.106117	0.369563	0.455872	0.524035	0.598363	0.71904
Pereira *et al.* (1985)	CD1	Female	0.453666	0.0963253	0.303665	0.384955	0.448654	0.516608	0.622827
		Male	0.650342	0.167153	0.37248	0.522933	0.660456	0.772229	0.914794
Schmahl (1988)	NMRI	Female	0.0511349	0.00593372	0.04167	0.0469975	0.0509134	0.0550801	0.061308
		Female	0.0813521	0.010729	0.0648204	0.0737127	0.0806609	0.0882508	0.100297
		Male	0.0819858	0.010706	0.0654327	0.0744036	0.0812874	0.088867	0.100923
		Male	0.113765	0.016685	0.0891173	0.101707	0.11222	0.124072	0.143985
Searle and Jones (1976)	A	Male & Female	0.102345	0.0307605	0.0492138	0.0821283	0.103482	0.123574	0.151421
	C57BL	Male & Female	0.246532	0.0422712	0.180748	0.217317	0.244314	0.273666	0.32047
	DBAF	Male & Female	0.123967	0.0202948	0.090089	0.110854	0.12422	0.13749	0.156851
	IF	Male & Female	0.118889	0.0283388	0.0747125	0.0992445	0.11737	0.137096	0.168199
Vesselinovitch *et al.* (1974)	$B6C3F_1$	Female (Day 1)[b]	0.0901191	0.0182086	0.0653432	0.0770816	0.0873993	0.100164	0.124208
		Female (Day 15)[c]	0.0555416	0.00590128	0.0463567	0.0513833	0.0552225	0.0593717	0.0658145
		Male (Day 1)[b]	0.0784357	0.0094572	0.0638592	0.0718073	0.0778351	0.0844925	0.0950637
		Male (Day 15)[c]	0.115803	0.0193859	0.0886015	0.102078	0.113324	0.126753	0.151878
	$C3AF_1$	Female	0.0162293	0.00195763	0.0130923	0.0148687	0.0161727	0.0175495	0.0195461
		Male (Day 1)[b]	0.0478472	0.00952346	0.03385	0.0410128	0.0468717	0.0537055	0.0652326
		Male (Day 15)[c]	0.0337552	0.00545562	0.0254094	0.0298703	0.0333879	0.0372465	0.0434698
Vesselinovitch (1983)	$B6C3F_1$	Female	0.0325201	0.00615214	0.0229783	0.0281148	0.0321294	0.0365301	0.0435769
		Male	0.0695924	0.0120803	0.0509478	0.0609427	0.0687729	0.0774048	0.0913846

[a] Day of sacrifice.
[b] Mice were dosed on day 1 of life.
[c] Mice were dosed on day 15 of life.

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Table E3. ENU Juvenile Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	Percentiles				
					5th	25th	50th	75th	95th
Vesselinovitch *et al.* (1973)	$B6C3F_1$	Female	0.00126335	0.000460445	0.000605155	0.000927673	0.00121074	0.00153788	0.00212734
		Male	0.00337468	0.000804246	0.00213171	0.00279749	0.00331756	0.00389027	0.00481319
Vesselinovitch *et al.* (1974)	$B6C3F_1$	Female	0.0463117	0.00618634	0.0367588	0.0419302	0.045902	0.0503072	0.0572276
		Male	0.0441913	0.00498978	0.0363954	0.04067	0.0439454	0.0474287	0.0529056
	$C3AF_1$	Female	0.00579571	0.00122561	0.00380638	0.00495406	0.00577705	0.00661704	0.00784915
		Male	0.00713611	0.00130036	0.00503552	0.00623303	0.00711547	0.00800999	0.0093149
Vesselinovitch (1983)	$B6C3F_1$	Female	0.00451849	0.00192539	0.00183844	0.00309386	0.00425096	0.00566614	0.00819086
		Male	0.00886785	0.00285617	0.00458294	0.00681412	0.00858931	0.0106642	0.0139855

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Equal Weighting of Potency Distributions, without or with Truncation.

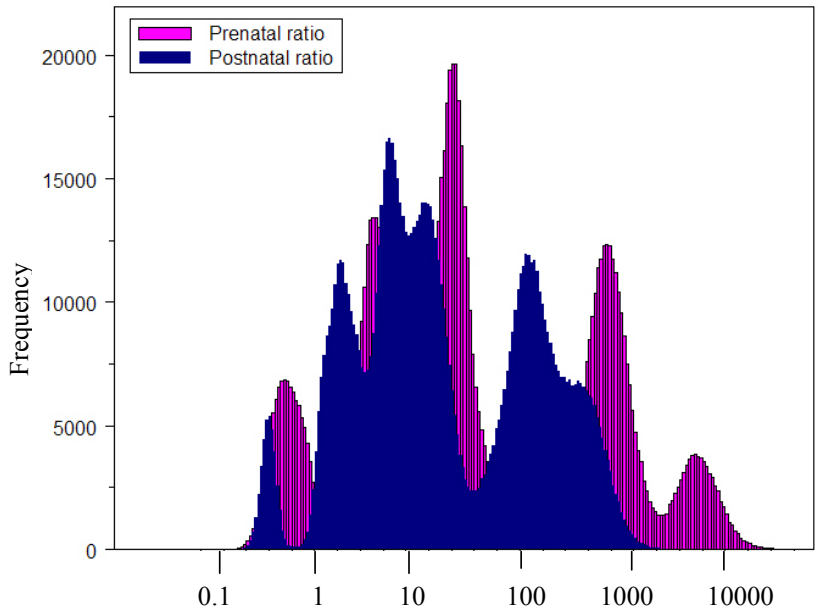
In the variation on Method 1, where the potency distributions derived from each experiment are truncated at the 5th and 95th percentiles, the results for the LP_j ratio distributions are not appreciably different from those obtained without the truncation, and indicate the same general conclusions (Table E4).

Table E4. ENU Prenatal and Postnatal LP_j Ratio Distributions – Equal Weighting of Potency Distributions
Method 1, as presented in the main text, and Method1 (truncated)

Percentiles	Method 1		Method 1 (truncated)	
	Prenatal LP_j Ratio	Postnatal LP_j Ratio	Prenatal LP_j Ratio	Postnatal LP_j Ratio
5th	0.53	1.14	0.53	1.18
10th	0.94	1.65	0.93	1.68
20th	3.86	3.03	3.89	3.00
30th	6.56	5.39	6.59	5.45
40th	11.60	8.09	11.63	8.07
50th	19.30	12.84	19.40	12.81
60th	27.13	21.87	26.66	20.76
70th	116.16	88.96	137.82	92.27
80th	679.56	154.90	687.33	152.78
90th	1266.12	325.80	1173.53	319.53
95th	4381.63	519.75	4557.69	506.81

Figure E-1 shows the ENU prenatal and postnatal LP_j ratio frequency distributions generated using Method 1. Both the prenatal and postnatal LP_j ratio frequency distributions are multi-modal. The ENU postnatal LP_j ratio distribution has a similar overall shape as the prenatal LP_j ratio distribution, with a shift to the left such that the values of the distribution are not as extreme. The ENU postnatal LP_j ratio distribution is more compact and lacks the most extreme values observed in the rightmost tail of the prenatal LP_j ratio distribution (Figure 21, in the main text), although large values in the upper tails are evident (See also Table E4). Table E4 shows the ENU LP_j ratios calculated using Method 1 barely differ when the potency distributions are truncated at the fifth and ninety-fifth percentiles to eliminate the extreme values, prior to developing the mixture potency distributions.

Figure E-1. Method 1 ENU Prenatal and Postnatal LPj Ratio Frequency Distributions – Equal Weighting of Potency Distributions



Alternative Weighting: Weighting Potency Distributions by Inverse-Variance and Interquartile Range.

The ENU prenatal and postnatal LP_j ratio distributions computed by Method 2a and Method 2b differ substantially from one another, as shown in Figure E-2. This is because each lifestage has a grouping of experiments that have narrower confidence intervals than the remaining grouping of experiments. Within each lifestage, those experiments with the narrowest confidence intervals are given greater weight. Figure E-2 demonstrates that the differences observed between the weighting methods is due to greater weight being assigned to these studies with the narrowest confidence intervals via the inverse-variance weighting method compared to the interquartile range weighting method.

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The ENU prenatal LP_i ratio distributions computed via Method 2a and 2b have medians equal to 3.81 and 11.05, respectively. The ENU postnatal LP_j ratio distributions computed via Method 2a and 2b have medians equal to 0.55 and 7.24, respectively. Clearly, the inverse-variance weighting results suggest less susceptibility from early life exposure to ENU than the interquartile range weighting results. The inverse-variance weighting scheme tends to weigh the studies with narrower distributions, and in the case of the ENU pre- and postnatal studies, smaller potency values, considerably more heavily as compared to interquartile range weighting.

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Both weighting methods clearly indicate greater inherent sensitivity of the prenatal lifestage to ENU, which was also observed when studies were weighted equally (Method 1). The two weighting methods (2a and 2b) yield strikingly different results for the postnatal lifestage, however. Using inverse variance weighting, approximately half of the ENU postnatal LPj ratio distribution is less than unity, indicating no substantial inherent sensitivity for the postnatal compared to juvenile lifestage. With interquartile weighting, the 10th percentile is 1.04 and half the distribution exceeds 7.0, indicating a strong postnatal sensitivity. The inverse variance results are also substantially different to the results seen when all studies are equally sampled, as shown in Method 1 above. However, the interquartile range weighting results are similar to those obtained via Method 1 though slightly more moderate. Results from both Method 2a and 2b indicate that prenatal sensitivity is substantially greater than postnatal sensitivity.

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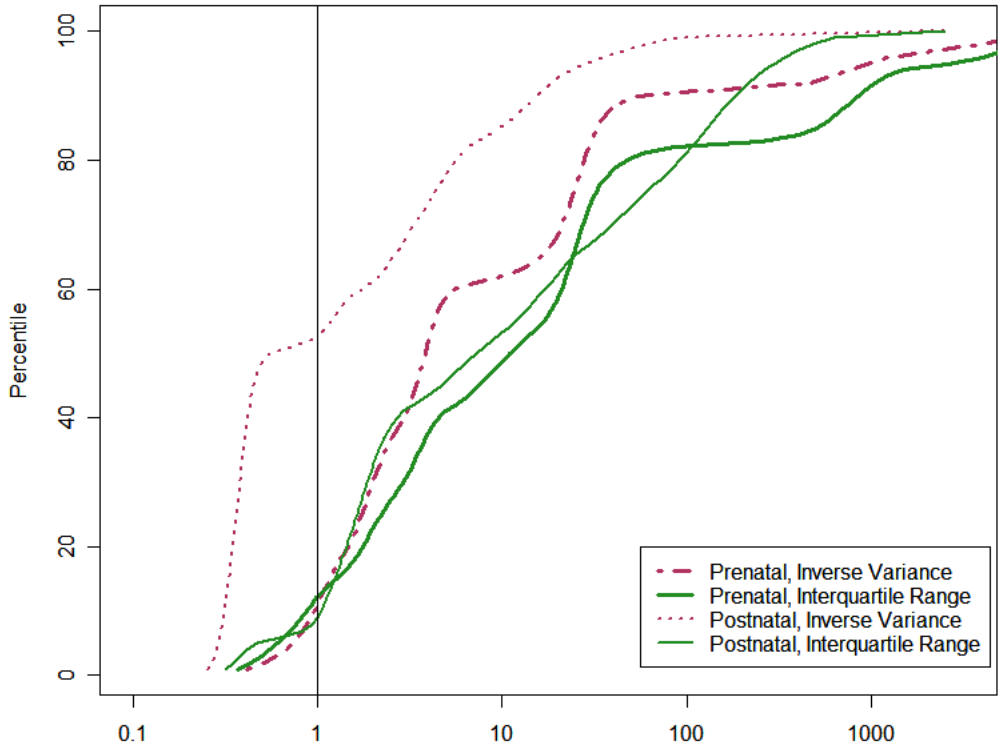
Figure E-2. Methods 2a and 2b ENU Prenatal and Postnatal LP_{ij} Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions



LP_{ij} Ratio

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Table 5a. Method 2 ENU Prenatal and Postnatal LPj Ratio Distributions – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal LP_i Ratio	Postnatal LP_i Ratio	Prenatal LP_i Ratio	Postnatal LP_i Ratio
5th	0.74	0.29	0.61	0.47
10th	0.95	0.31	0.87	1.04
20th	1.45	0.35	1.75	1.43
30th	1.98	0.38	2.91	1.85
40th	2.93	0.42	4.55	2.69
50th	3.81	0.55	11.05	7.24
60th	5.45	1.72	20.97	17.05
70th	21.18	3.33	27.36	39.81
80th	27.75	5.61	47.64	91.56
90th	53.70	15.32	852.11	182.93
95th	940.28	27.92	2608.68	296.87

Table 5b. Method 2 ENU Prenatal and Postnatal ASFj Distributions – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal ASF_i	Postnatal ASF_i	Prenatal ASF_i	Postnatal ASF_i
5th	2.22	0.78	1.83	1.36
10th	2.85	0.84	2.61	3.02
20th	4.35	0.94	5.25	4.15
30th	5.94	1.03	8.73	5.37
40th	8.79	1.13	13.65	7.80
50th	11.43	1.48	33.15	21.00
60th	16.35	4.64	62.91	49.45
70th	63.54	8.99	82.08	115.45
80th	83.25	15.15	142.92	265.52
90th	161.1	41.36	2556.33	530.50
95th	2820.84	75.38	7826.04	860.92

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Early Life Across-Lifestage Exposure Studies of Two Non-Genotoxic Carcinogens

Early in life studies in which treatment group exposures crossed multiple lifestages were excluded from the main analyses presented in this document, as across-lifestage exposures preclude derivation of age-at-exposure sensitivity measures for specific early lifestages. Some studies with early life across-lifestage exposures have been included in the analyses of Barton *et al.* (2005), and can provide information on early life vs. later life sensitivity. This appendix presents the lifestage potency (LP) ratio distribution statistics derived from analyses of experiments conducted in mice with two non-genotoxic carcinogens: diphenylhydantoin (Chhabra *et al.*, 1993a) and polybrominated biphenyls (Chhabra *et al.*, 1993ab). In these studies separate groups of animals were exposed to either diphenylhydantoin or polybrominated biphenyls across multiple "early life" lifestages (i.e., prenatal, postnatal and juvenile) or during the adult lifestage. For the early lifestage exposure groups, exposures began prior to conception, and continued throughout the prenatal, postnatal, and post-weaning periods, up to the age of eight weeks.

Table F1 presents the LP ratio distributions and study details for these early life across-lifestage datasets.

Table F1. Across-Lifestage Exposure Studies: Estimated Lifestage Potency Ratios for Two Non-Genotoxic Chemicals

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Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Diphenylhydan-toin	Chhabra *et al.* (1993a)	Mouse	$B6C3F_1$	Female	No	2	2.14E+01	0.000%	2.46E+00	1.25E+01	2.00E+01	2.87E+01	4.42E+01
Polybrominated biphenyls	Chhabra *et al.* (1993b)	Mouse	$B6C3F_1$	Female	No	2	3.10E+00	0.000%	1.59E+00	2.36E+00	2.99E+00	3.72E+00	4.96E+00
				Male	No	2	3.90E+00	0.000%	1.93E+00	2.85E+00	3.68E+00	4.72E+00	6.62E+00

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adjusted to take into account the longer period for cancer to manifest after early life exposures, range from

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.6 to 37.1, depending on the method used to combine studies

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– 42.4, depending on the method used to combine studies

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increased susceptibility appears even more pronounced once adjustments are made to take into account the long period cancer has to manifest when exposure occurs early in life. M

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to 9.4, depending upon the method used to combine studies

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Method 2a − The potency distributions were sampled based upon weights equal to the computed inverse-variance of each (logarithm) potency

distribution. That is, the variance is calculated for the distribution of the logarithm of the q_1, Var[log q_1]. The likelihood that an q_1 is sampled is proportional to 1/Var(log[q_1]).

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Figure 6 Legend

1. Vesselinovitch *et al*. (1979a), mouse, B6C3F$_1$, F, day -9 to 21
2. Ibid, M, day -9 to 21
3. Zeller *et al*. (1978), rat, Sprague Dawley, M/F day -2
4. Turusov *et al.* (1992), mouse, CBA, F, day -2
5. Mohr et al. (1975), hamster, Syrian Golden, day -15 to -1
6. Mohr *et al*. (1995), hamster, Syrian Golden, F, day -3
7. Althoff *et al*. (1977), hamster, Syrian Golden, M/F, day -9 to -3
8. Ibid, day -9 to -3
9. Althoff and Grandjean (1979), hamster, Syrian Golden, F, day -9 to -3
10. Druckrey and Landschutz (1971), rat, BD IX, M/F, day -10
11. Ibid, day -3
12. Naito *et al*. (1981), rat, Wistar, day -9
13. Ibid, day -9
14. Tomatis *et al*. (1977), rat, BDVi, F, day -5
15. Althoff and Grandjean (1979), hamster, Syrian Golden, M/F, day -9 to -3
16. Tomatis *et al*. (1971), mouse, CF-1, F day -4 to -1
17. Turusov *et a*l. (1973), mouse, CF-1, F, day -2
18. Anderson *et al.* (1989), mouse, C3H & B6C3 F$_1$,M/F day -8 to -4
19. Vesselnovitch *et al.* (1979a), mouse, B6C3 F$_1$, M, day -9 to -3
20. Vesselnovitch *et al.* (1979b), mouse, B6C3 F$_1$, F day -9 to -3
21. Choudari Kommineni *et al*. (1970), rat, MRC, M/F, day -4
22. Maltoni *et al.* (1981), rat, Sprague Dawley, M/F day -13 to -7

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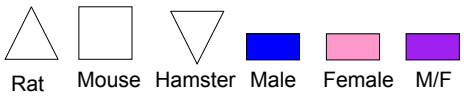

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LP ratio calculation is based on juvenile potency distribution)

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The cumulative distribution functions for Method 1 and Method 2 are nearly identical up to the 70th percentile. After the 70th percentile, Method 2 has slightly larger values as compared to Method 1. The most compact postnatal ASF distributions generally have

values that are significantly greater than unity. As a result, the inverse-variance method (Method 2) produces a mixture cumulative distribution that is shifted slightly to the right of the distribution derived using Method 1, where equal weighting is given to all studies within a chemical. The magnitude of this rightward shift with Method 2 is not particularly large however because there were no single studies amongst those chemicals with multiple studies with considerably smaller variances than the others in the set. The postnatal ASF mixture cumulative distribution derived via Method 3 has percentile values that are considerably larger than the other methods beyond the 5th percentile. The most peaked mode of the postnatal ASF mixture frequency distribution is similar across the mixing algorithms employed (i.e., Methods 1-3). However, when a single study with the largest median value is selected to represent the chemical (Method 3), the percentiles of the distribution become somewhat larger as compared to that seen using Methods 1 or 2.

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n the results for the postnatal multi-window analysis by the three methods are given

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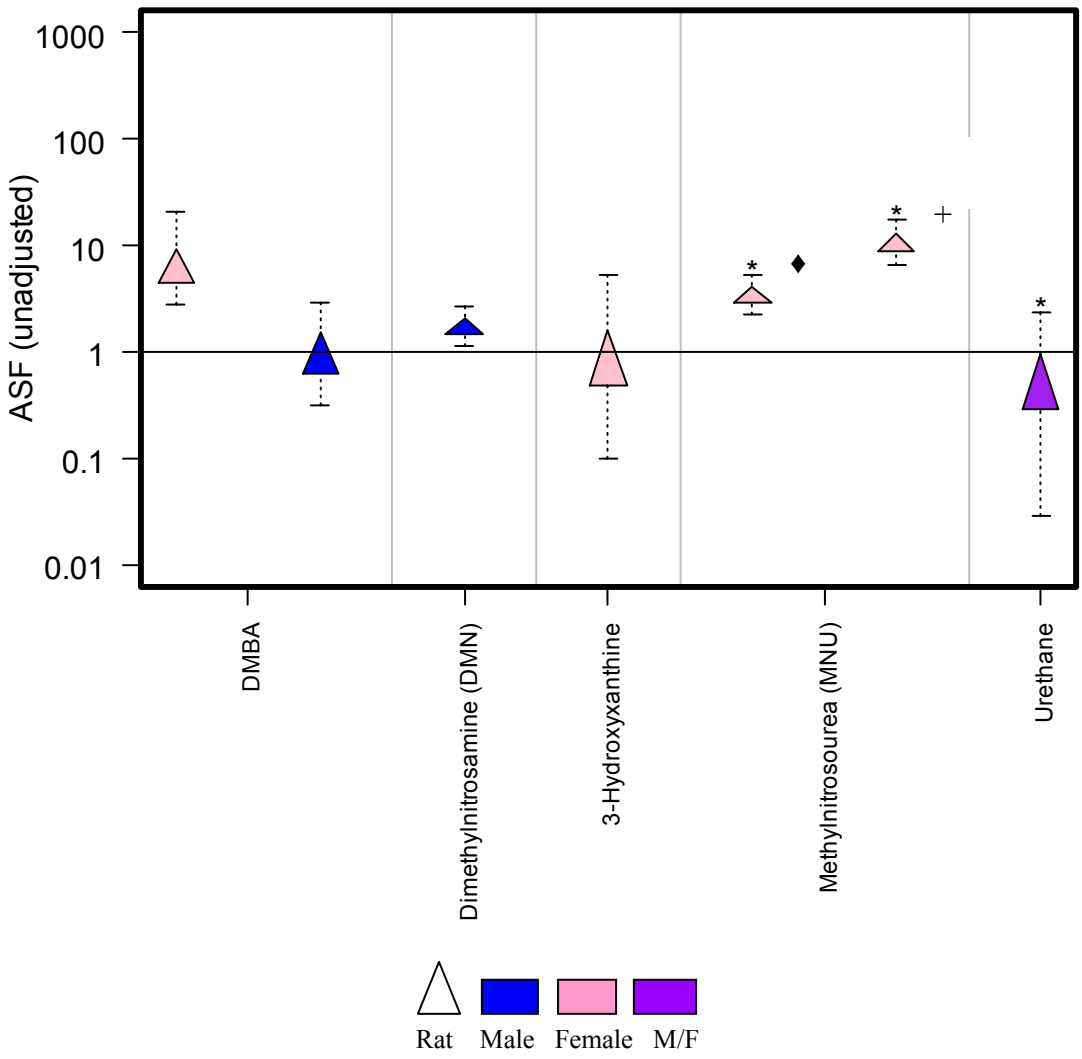

♦ Adult comparison group dosed on days 80-87
+ Adult comparison group dosed on days 140-147
* Adult comparison group dosing began during late juvenile

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lifestage (day 46) and continued through day 61

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Percentiles	**Method 1**	

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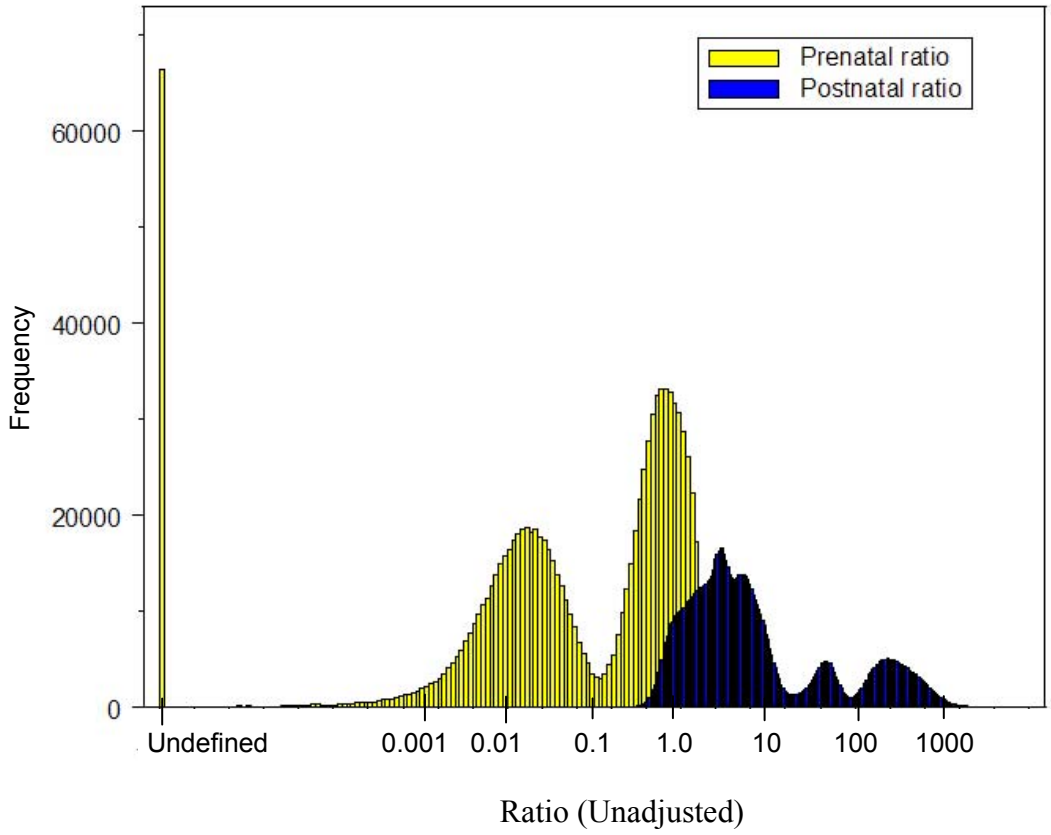
Figure 15 shows the DEN prenatal and postnatal ratio frequency distributions generated using Method 1. Both the prenatal and postnatal ratio frequency distributions are multi-modal.

Figure 15. Method 1 DEN Prenatal and Postnatal Ratio Frequency Distributions –Equal Weighting of Potency Distributions



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Method 2: Weighting Potency Distributions by Inverse-Variance and the Interquartile Range.

Figure 16 shows the DEN prenatal and postnatal ratio cumulative distribution functions generated using Method 2a, weighting by inverse-variance, and Method 2b, weighting by the interquartile range (IQR). Qualitatively the results are similar to Method 1, with considerable sensitivity exhibited in the postnatal window. The magnitude of the differences in the ratio distributions for DEN across the prenatal and postnatal exposure windows is evident.

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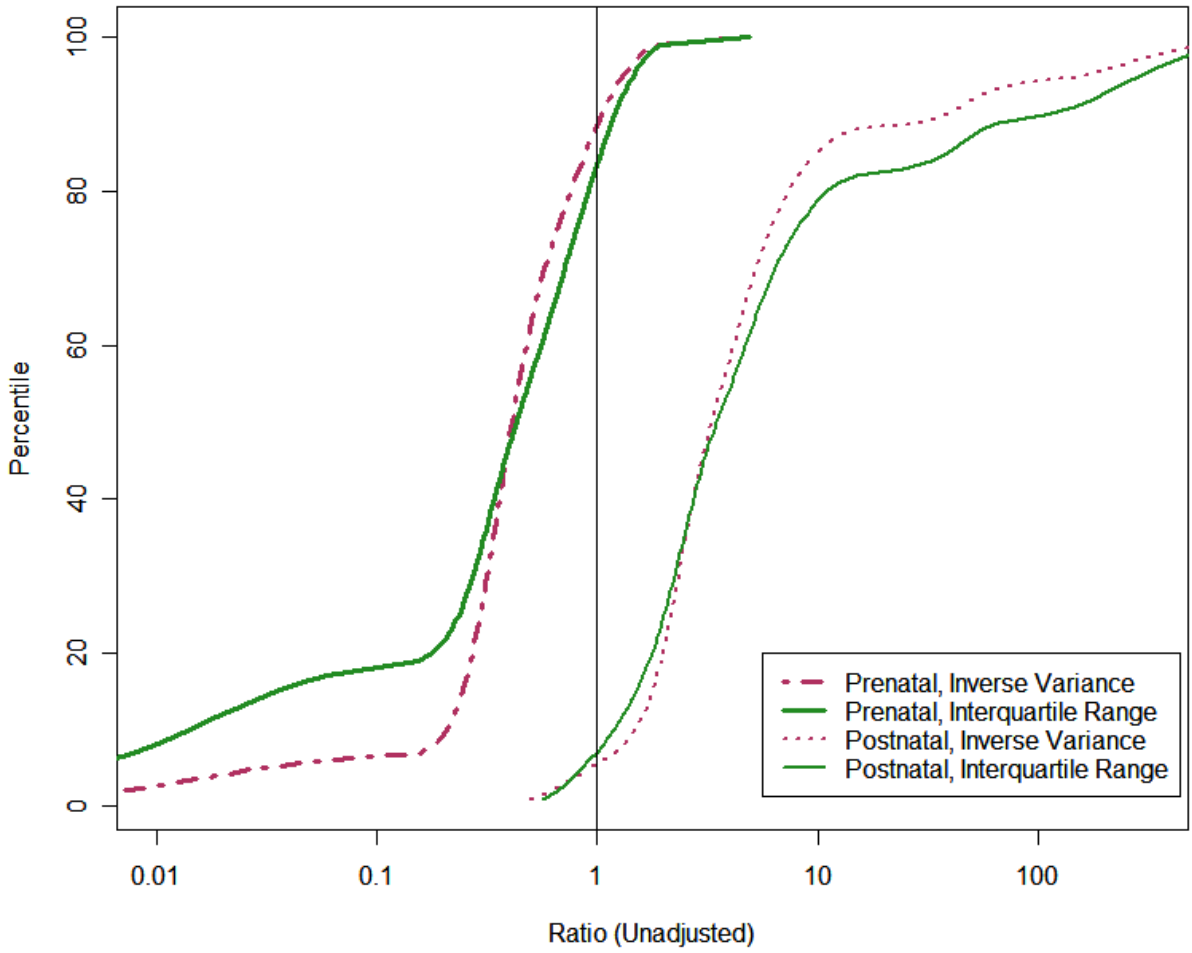
Figure 16. Methods 2a and 2b DEN Prenatal and Postnatal Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions



The percentiles for the prenatal and postnatal ratio distributions are provided in Table 11a (unadjusted) and b (adjusted for early versus adult timing of exposure). With inverse-variance weighting, slightly less than 89% of the unadjusted prenatal ratio distribution lies below the value of one. Although not statistically significant, the distributional statistics suggest that mice exposed during the prenatal age window are less prone to the tumorigenic effects of DEN as compared to those exposed as juveniles. For the unadjusted postnatal ratio distribution, more than 94% of the unadjusted postnatal ratio distribution is greater than unity under Method 2a (inverse-variance weighting),

indicating that mice exposed during the postnatal age window are more prone to the tumorigenic effects of DEN than those exposed as juveniles. The distributional differences in cancer risk (as compared to juveniles) between DEN exposures occurring during a prenatal window versus a postnatal window are quite evident.

Table 11a. Method 2 DEN Prenatal and Postnatal Ratio Distributions (Unadjusted)– Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.03	0.93	0.005	0.85
10th	0.21	1.49	0.01	1.19
20th	0.27	1.99	0.18	1.77
30th	0.32	2.34	0.27	2.24
40th	0.36	2.74	0.34	2.73
50th	0.41	3.31	0.43	3.48
60th	0.47	4.18	0.55	4.71
70th	0.58	5.27	0.71	6.42
80th	0.75	7.36	0.91	11.02
90th	1.04	37.80	1.20	106.70
95th	1.30	154.34	1.45	287.68

Table 11b. Method 2 DEN Prenatal and Postnatal Ratio Distributions (Adjusted*)– Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.09	2.69	0.02	2.47
10th	0.62	4.33	0.03	3.45
20th	0.81	5.78	0.54	5.13
30th	0.95	6.80	0.81	6.50
40th	1.08	7.94	1.02	7.92
50th	1.23	9.60	1.29	10.09
60th	1.42	12.12	1.65	13.66
70th	1.73	15.27	2.13	18.62
80th	2.25	21.35	2.73	31.96
90th	3.13	109.62	3.60	309.43
95th	3.90	447.59	4.35	834.27

*Adult vs. early-life timing of exposure adjustment

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Percentiles	Method 1	Method 1 (truncated)

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Figure 19 shows the ENU prenatal and postnatal ratio frequency distributions generated using Method 1. Both the prenatal and postnatal ratio frequency distributions are multi-modal. The ENU postnatal ratio distribution has a similar overall shape as the prenatal ratio distribution, with a shift to the left such that the values of the distribution are not as extreme. The ENU postnatal ratio distribution is more compact and lacks the most extreme values observed in the rightmost tail of the prenatal ratio distribution (Figure 18), although large values in the upper tails are evident (See also Table 12). Table 12 shows the ENU ratios calculated using Method 1 barely differ when the potency distributions are truncated at the fifth and ninety-fifth percentiles to eliminate the extreme values, prior to developing the mixture potency distributions.

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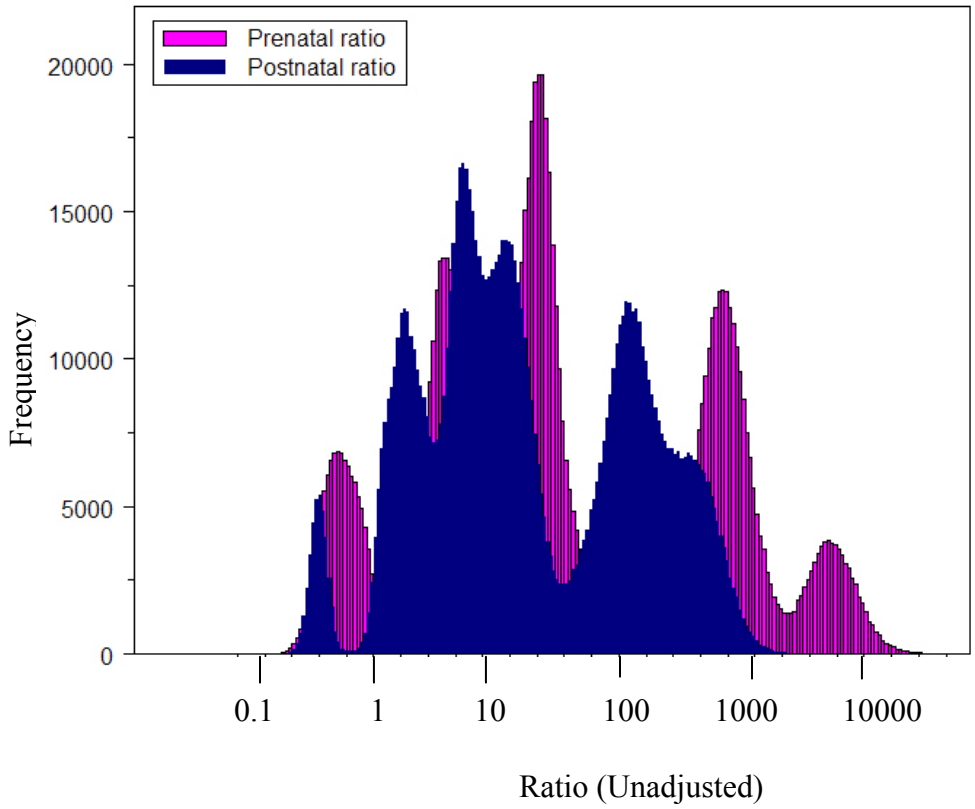
Figure 19. Method 1 ENU Prenatal and Postnatal Ratio Frequency Distributions – Equal Weighting of Potency Distributions



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ratios calculated using Method 1, after adjustment is made for early versus adult timing of exposure.

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with Adult vs. Early-Life Timing of Exposure Adjustment

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Discussion

Data from studies on 23 unique carcinogens, 20 of which are considered to act via primarily genotoxic modes of action, were analyzed. Of these 20 carcinogens, 15 are thought to require metabolic activation to the ultimate carcinogenic species. The analyses indicate that both the prenatal and postnatal lifestages can be much more susceptible to developing cancer than the adult lifestage. As an index of inherent susceptibility, one that does not account for the longer time early exposures can manifest,

an LP ratio was derived. This index compares the carcinogenicity activity when exposures occur early in life compared to older ages, for the same period of time between initial exposure and observation of effect. For the multi-lifestage exposure studies, the median LP ratio for the postnatal period was 4.6 or 7.5, and the upper 95% confidence bound ranged from 123 to 188, depending on the method of combining the ASF distributions underlying studies on the same chemical.

There were few cases of LP ratios less than 1.0 for the postnatal lifestage. These results indicate that in general, for the chemicals studied, there is inherently greater susceptibility during the early postnatal compared to the adult period. The differences between postnatal and adult susceptibility appear more pronounced once the longer period of time that exposed young have to develop tumors is addressed by taking into account time-of-dosing, in calculating the ASF. The median value for the postnatal ASF indicates for the chemicals studied here a 13.5- fold greater contribution to lifetime cancer risk when exposure occurs during this period, compared to the same exposure averaged throughout the adult period; the upper 90^{th} percentile ASF was 211. The DEN and ENU case studies also exhibited substantial sensitivity in the postnatal lifestage, with inherent susceptibility about half an order of magnitude greater than juveniles for DEN, and about an order of magnitude greater than juveniles for ENU, and again greater susceptibility once the longer period of time that exposed young have to develop tumors is ttaken into account.

Regarding *in utero* exposure, few studies provided data indicative of equal inherent adult and prenatal susceptibility, with an LP ratio of unity. For the multi-lifestage exposure studies, the prenatal LP ratio distributions are roughly bimodal, with LP ratios for several studies significantly greater than unity and several others significantly less than unity (Figure 6). The median LP ratio mixture distribution was 2.5. The median estimate of the prenatal ASF was 2.9, and the mean estimate ws 21.1. This modality in the prenatal LP ratio and ASF mixture distributions is reflected in the case studies. The prenatal LP_j ratio for DEN has a median of 0.1, and the majority of the distribution falls below unity. This is suggestive of reduced inherent susceptibility *in utero*. In contrast the median prenatal LP_j ratio for ENU was 19.3, with the majority of the distribution exceeding

unity, indicative of greater inherent *in utero* susceptibility. In considering implications of the DEN and ENU case studies it is important to recognize that the referent groups were juvenile rather than adult animals. The prenatal (and postnatal) LP_j ratios and ASF_js are likely to be underestimates, to the extent that some of the apparent sensitivity for DEN and ENU in the early postnatal period carries through to the juvenile period.

ENU is a direct acting carcinogen that does not require metabolic activation to alkylate DNA, forming DNA adducts and mutations that ultimately result in the formation of tumors (Slikker III *et al.,* 2004). In contrast, DEN requires metabolic activation by cytochrome P450 enzymes (e.g., P450 2E1, P450 2A6) to form the active DNA ethylating species (Brittebo *et al.*, 1981). While both ENU and DEN cross the placenta and are widely distributed in fetal tissues (Rice *et al.* 1989; Brittebo *et al.*, 1981), DEN can not be metabolized to any significant extent by fetal tissues until relatively late in gestation (i.e., gestation day 18 in the mouse), and after birth the expression of P450 2E1 progressively increases, reaching adult levels by day 30 (Brittebo *et al.*, 1981). This may explain the lower fetal susceptibility of DEN. However, the multi-lifestage exposure studies illustrate that *in utero* metabolic status is not the sole determinant of *in utero* susceptibility: benzidine and safrole require metabolic activation and exhibit greater susceptibility from prenatal exposure (see Figure 6).

There are just five chemicals and seven studies, two of which are not independent (i.e., the MNU studies of Grubbs *et al*., 1983), available to examine susceptibility in the juvenile lifestage. The LP ratio distributions indicate significantly greater inherent susceptibility in this period for three of the independent studies, with the three remaining independent studies consistent with equal inherent susceptibility to adult animals (Figure 14). For the juvenile lifestage, the ASF mixture distribution was 4.5 at the 50^{th} percentile and 19.7 at the 95^{th} percentile.

The studies that comprise the set of multi-lifestage exposure studies available for these analyses were not homogeneous. That is, they do not represent observations from the same distribution. Sensitivity analyses were conducted to test the robustness of the

findings to different procedures for analyzing data and combining results. Of the methods used to combine the LP ratio distributions for underlying studies within each lifestage, the method of equally weighting studies within a chemical appears to best represent the available data. The use of inverse variance in weighting LP ratio distributions within a chemical may underweight small studies and overweight large ones, and thus produce a LP ratio mixture distribution that does not accurately reflect the overall data. This is clearly illustrated by the results of the postnatal ENU case study analyses. The method of selecting a single study (i.e., that with the largest median LP ratio) to represent each chemical may also result in inadvertent bias if a selected study is not representative of the group being studied.

In taking into account the longer period of time for early carcinogen exposures to manifest, the hazard function was assumed to increase with the third power of age. If the true rate of increase with age is greater than that, then the ASFs presented here may result in underestimates of the true sensitivity of these early lifestages.

As the multi-lifestage exposure and chemical-specific case studies show, there appears to be considerable variability in age-at-exposure related susceptibility across carcinogens. There is also variability in age-at-exposure related susceptibility among studies of the same carcinogen. The sources of variability evident in the analyzed studies include timing of exposure within a given lifestage, and gender, strain, and species differences in tumor response. The set of studies identified and analyzed was not sufficiently robust to fully describe quantitatively the variability. This variability raises concerns that selection of the median (the 50^{th} percentile) estimates may considerably underestimate effects for certain carcinogens or population groups. Relatively large variability in humans in response to carcinogens is expected to be common (Finkel, 1995; 2002).

Several of the carcinogens studied induced tumors at multiple sites in the same experiment, and at different sites, depending upon the lifestage during which exposure occurred. The cancer potencies used in the early vs. later life comparisons were based on all treatment-related tumors. When treatment-related tumors were induced at multiple

sites in the same experiment, or at the same site, but arising from different cell types, the slopes of the dose response curves from these different tumor sites or types were statistically combined to create an overall multisite cancer potency distribution for that experiment. The result reflects the total cancer impact associated with the carcinogen exposure in question. This approach differs from other researchers investigating early vs. late in life differences (e.g., Barton *et al.*, 2005; Hattis *et al.*, 2004; 2005). We believe this provides a more complete approach for considering age specific differences in carcinogenic activity.

One limitation of the approach was the focus on lifestages, without attempting to describe changes in susceptibility that occur within a lifestage. Timing of carcinogen exposure within a given lifestage can affect the cancer outcome observed. This is illustrated by experiments with 1-ethyl-1-nitrosobiuret in prenatal and adult rats by Druckrey and Landschutz (1971). A three fold difference in activity was observed between two prenatal exposure groups, one exposed on prenatal day -10 and the other on prenatal day -3 (See Figure 6 and Appendix B, Table B1). The timing of exposure within the adult age window can also affect the cancer outcome, as illustrated by the experiments of Grubbs *et al.* (1983), in which female rats exposed early in the adult period (days 80 through 87) were more than three times as sensitive to the breast cancer effects of MNU than females exposed six weeks later (Figure 14 and Appendix B, Table B3). In general the adult comparison groups in the multi-lifestage exposure studies were fairly young. The extent to which this may result in an overall bias of the results presented here is unclear. Also for several cases, juvenile animals were used as the later life exposure group. In these cases the ASFs are likely underestimates of the relative sensitivity of the prenatal and postnatal lifestages, compared to that of the adult lifestage.

Excluded from the analysis presented here were early in life studies in which exposure of a given exposure group crossed multiple lifestages. An example of results from studies of this type is provided by mouse studies for two non-genotoxic carcinogens, diphenylhydantoin (Chhabra *et al.*, 1993a) and polybrominated biphenyls (Chhabra *et al.*, 1993ab), in which exposures began prior to conception, and continued throughout the

prenatal, postnatal, and post-weaning periods, up to the age of eight weeks. The data, shown in Appendix F, demonstrate an increased sensitivity associated with exposures to either of these non-genotoxic carcinogens during the entire early life period, as compared to exposures during only the adult lifestage. Some studies that crossed multiple lifestages were included in the analyses of Barton *et al.* (2005), which are consistent with the general conclusions here.

Barton *et al.* (2005) discussed data on 18 unique carcinogens, but ultimately analyzed data on six mutagenic carcinogens (benzidine, diethylnitrosamine, 3-MC, safrole, urethane, and vinyl chloride) to derive the age dependent adjustment factor of 10 for carcinogen exposures occurring between birth and the second birthday, as specified in the U.S. EPA's (U.S. EPA, 2005) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*. In performing the analysis, Barton *et al.* (2005) compared tumor site-specific potencies, while

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Method 2: Weighting Potency Distributions by Inverse-Variance and Interquartile Range. The ENU prenatal and postnatal ratio distributions computed by Method 2a and Method 2b differ substantially from one another, as shown in Figure 20. This is because each exposure window has a grouping of experiments that have narrower confidence intervals than the remaining grouping of experiments. Within each exposure window, those experiments with the narrowest confidence intervals are given greater weight. Figure 20 demonstrates that the differences observed between the weighting methods is due to greater weight being assigned to these studies with the narrowest

confidence intervals via the inverse-variance weighting method compared to the interquartile range weighting method.

The ENU prenatal ratio distributions computed via Method 2a and 2b have medians equal to 3.81 and 11.05, respectively. The ENU postnatal ratio distributions computed via Method 2a and 2b have medians equal to 0.55 and 7.24, respectively. Clearly, the inverse-variance weighting results suggest less susceptibility from early life exposure to ENU than the interquartile range weighting results. The inverse-variance weighting scheme tends to weigh the studies with narrower distributions, and in the case of the ENU pre- and postnatal studies, smaller potency values, considerably more heavily as compared to interquartile range weighting.

Both weighting methods clearly indicate greater inherent sensitivity of the prenatal window to ENU, which was also observed when studies were weighted equally (Method 1). The two weighting methods (2a and 2b) yield strikingly different results for the postnatal window, however. Using inverse variance weighting, approximately half of the ENU postnatal ratio distribution is less than unity, indicating no substantial inherent sensitivity for the postnatal compared to juvenile development window. With interquartile weighting, the 10^{th} percentile is 1.04 and half the distribution exceeds 7.0, indicating a strong postnatal sensitivity. The inverse variance results are also substantially different to the results seen when all studies are equally sampled, as shown in Method 1 above. However, the interquartile range weighting results are similar to those obtained via Method 1 though slightly more moderate. Results from both Method 2a and 2b indicate that prenatal sensitivity is substantially greater than postnatal sensitivity.

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Figure 20. Methods 2a and 2b ENU Prenatal and Postnatal Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions

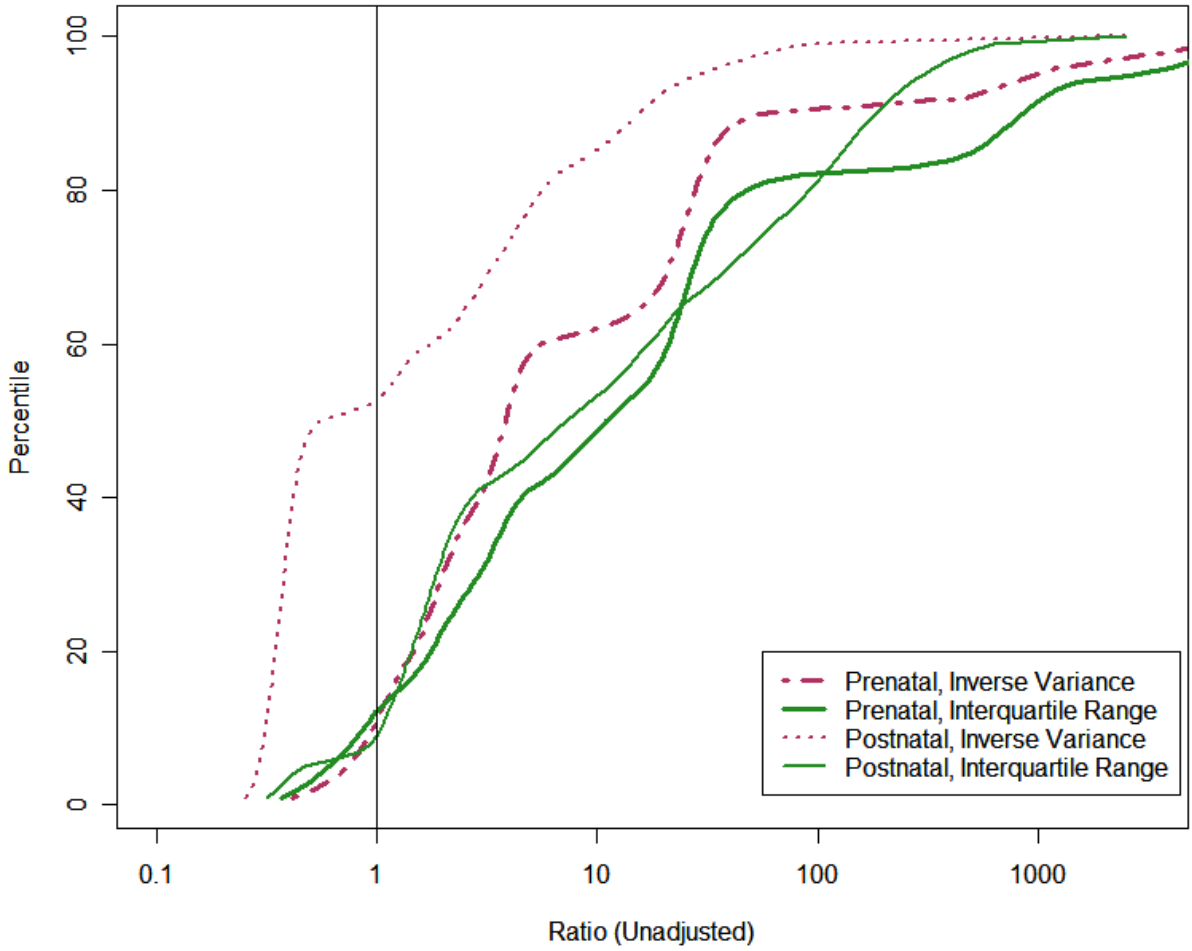

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Table 14a. Method 2 ENU Prenatal and Postnatal Ratio Distributions (Unadjusted)–Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.74	0.29	0.61	0.47
10th	0.95	0.31	0.87	1.04
20th	1.45	0.35	1.75	1.43
30th	1.98	0.38	2.91	1.85
40th	2.93	0.42	4.55	2.69
50th	3.81	0.55	11.05	7.24
60th	5.45	1.72	20.97	17.05
70th	21.18	3.33	27.36	39.81
80th	27.75	5.61	47.64	91.56
90th	53.70	15.32	852.11	182.93
95th	940.28	27.92	2608.68	296.87

Table 14b. Method 2 ENU Prenatal and Postnatal Ratio Distributions (Adjusted*) – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	2.22	0.78	1.83	1.36
10th	2.85	0.84	2.61	3.02
20th	4.35	0.94	5.25	4.15
30th	5.94	1.03	8.73	5.37
40th	8.79	1.13	13.65	7.80
50th	11.43	1.48	33.15	21.00
60th	16.35	4.64	62.91	49.45
70th	63.54	8.99	82.08	115.45
80th	83.25	15.15	142.92	265.52
90th	161.1	41.36	2556.33	530.50
95th	2820.84	75.38	7826.04	860.92

*Adult vs. early-life timing of exposure adjustment

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an adjustment is made to the ASF to take into account the longer period cancer has to manifest when exposure occurs early in life.

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to 307.6, depending on the method for combining the ASF distributions for the underlying studies

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, depending on the method used to combine studies on the same chemical

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adjusted to take into account the longer period for cancer to manifest for early life exposures, median estimates range from